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The synopsis part of this thesis is available as a pdf-file for download from the DTU research database ORBIT: http://www.orbit.dtu.dk.

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Preface

The work reported in this PhD thesis, entitled "Integrated assessment of chemical stressors and ecological impact in mixed land use stream systems", was conducted primarily at the Department of Environmental Engineering at the Technical University of Denmark from November 2013 to April 2017; an external stay at the Department of Bioscience – Stream and Wetland Ecology, Aarhus University was included (November 2014 to April 2015).

The research was carried out under the supervision of Professor Poul Løgstrup Bjerg and Associate Professor Ursula S. McKnight. Postdoctoral Researcher Jes Rasmussen, Aarhus University acted as informal co-supervisor regarding field campaigns, characterization of macroinvertebrates and statistical data evaluation of ecological impact.

The project was funded by GEOCON, Advancing GEOlogical, geophysical and CONtaminant monitoring technologies for contaminated site investigation (Innovation Fund Denmark), the Danish Environmental Protection Agency, the Region of Southern Denmark and the Technical University of Denmark.

The content of the PhD thesis is based on three scientific journal papers. The articles were prepared in collaboration with international researchers from Denmark and Germany. At the time of writing, one of the journal papers has been published, one is submitted and one is a manuscript in preparation for submission as listed:

I. Rasmussen, J.J., McKnight, U.S., Sonne, A.Th., Wiberg-Larsen, P., Bjerg, P.L., 2016. Legacy of a Chemical Factory Site: Contaminated Groundwater Impacts Stream Macroinvertebrates. Arch. Environ. Contam. Toxicol. 70, 219-230.

II. Sonne, A.Th., McKnight, U.S., Rønde, V., Bjerg, P.L., 2017. Assessing the spatial chemical contamination dynamics of a mixed land-use stream system. Submitted.

III. Sonne, A.Th., Rasmussen, J., Höss, S., Traunspurger, W., Bjerg, P.L., McKnight, U.S., 2017. Re-thinking stressor interaction: novel insights advancing stream ecosystem impact assessments. Manuscript.

The thesis is organized in two parts: the first part places into context the findings of the PhD in an introductory review; the second part consists of the papers listed above. These will be referred to in the text by their paper number written with the Roman numerals **I-III**.

Additionally, the following reports and publications, related to the topic of the thesis, have been co-authored during the PhD-study, and will also be referred to in the thesis:

Aisopou, A., Bjerg, P.L., Sonne, A.T., Balbarini, N., Rosenberg, L., Binning, P.J., 2015. Dilution and volatilization of groundwater contaminant discharges in streams. Journal of Contaminant Hydrology 172, 71-83.

Bjerg, P.L., Sonne, A.Th., Tuxen, N., Nielsen, S.S., Roost, S., 2014 (in Danish). Risk assessment of old landfills' impact on surface water (Risikovurdering af lossepladsers påvirkning af overfladevand). Orbicon A/S, Region Midtjylland, Region Syddanmark, Danish EPA, project no. 1604.

Nielsen, S.S., Tuxen, N., Frimodt, O., Bjerg, P.L., Sonne, A.Th., Binning, P.J., Fjordbøge, A.S., Aabling, J. 2014 (in Danish). Risk assessment of surface water impacted by point source related contaminated groundwater (Risikovurdering af overfladevand, som er påvirket af punktkildeforurenet grundvand). Danish EPA, project no. 1575.

Rønde, V., McKnight, U.S., Sonne, A.Th., Devlin, J.F., Bjerg, P.L., 2017. Contaminant mass discharge to streams: comparing direct groundwater velocity measurements and multi-level groundwater sampling with an in-stream approach. Submitted.

Kgs. Lyngby, June 2017

Anne Thobo Sonne

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This PhD project was accomplished with the consistent support, advice and great enthusiasm of my supervisors Poul L. Bjerg and Ursula S. McKnight from DTU Environment, and the informal supervisor Jes J. Rasmussen, Aarhus University. I am also grateful to all of the co-authors who contributed to my work. In particular the collaboration with Sebastian Höss, from Ecossa and the University of Bielefeld in Germany, and Walter Traunspurger, also from the University of Bielefeld, on the meiofauna has been very inspiring. It was a great pleasure to work with you.

I would further like to recognize those key persons who assisted in a technical manner and supported the field work, which otherwise would not have been successful: the DTU technicians Bent Skov, Jens Schaarup Sørensen, Flemming Møller, Flemming Thomsen and Erik Rønn Lange; and the technicians from Aarhus University: Tommy Silberg and Lise Lauridsen! I thank everyone involved in laboratory analyses for their assistance and kind support: Sinh Nygen, Mikael E. Olson, Christina M. Hagberg, Hanne Bøggild (DTU environment) and Jonny Nielsen (Aarhus University).

Furthermore I would like to thank the Region of Southern Denmark, Jørn K. Pedersen and Lone Dissing, and Central Denmark Region, Henrik R. Larsen, for their support to access previous studies and conduct field work.

I would like to point out my external stay at the Department of Bioscience – Stream and Wetland Ecology, Aarhus University (November 2014 to April 2015) with Jes J. Rasmussen and Anette Baattrup-Pedersen. I am very grateful for the support and the hospitality during multiple visits.

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Sincerely

Anne Thobo Sonne

Kgs. Lyngby, June 2017

Summary

The increasing human population and development pressure during the last century has motivated land use changes of importance on a global scale. Urban expansion and increasing agricultural production have thus created a wide range of pressures, which in particular affect the freshwater bodies such as streams, as they are highly connected to their catchment through their draining system. The pressures include hydromorphological alterations, as well as diffuse chemical sources (e.g. geogenic, agricultural activities) and point sources (e.g. wastewater outlets, contaminated sites). The degradation of these mixed land use stream systems causes critical changes and thus jeopardizes the health of the stream ecosystems.

The various chemical sources result in a highly diverse group of chemical stressors leading to a decrease in the chemical quality of the different stream compartments (i.e. stream water, hyporheic zone and bed sediment). These compartment(s) will be impacted differently by the various chemicals present in the system, depending on e.g. the stressor's pathway to the stream, their physico-chemical properties, and controlling hydrological and biogeochemical processes. The resulting impairment of the different stream compartments thus comprises both temporal and spatial variation. Despite the growing understanding of the complexity, approaches for a holistic risk assessment of the potential impacts in the three stream compartments of a mixed land use stream system are still missing.

To investigate and improve the understanding of the presence of multiple chemical stressors and any related ecological impacts in such a system, Grindsted stream was chosen as the study site for this PhD project. The groundwater-fed stream is situated in a mixed land use catchment hosting both urban and agricultural activities, including contaminated sites. To determine potential impacts, the chemical quality of both organic (i.e. pharmaceuticals, gasoline constituents, chlorinated solvents, and pesticides) and inorganic (i.e. metals, general water chemistry and macroions) compounds was assessed in all three stream compartments. To evaluate the magnitude of the sources, a combination of three established approaches was employed: contaminant mass discharge, toxic potential and threshold values. To subsequently account for potential ecological impacts, benthic invertebrate communities (both macro- and meiofauna) were characterized to enable a full coverage of the quality of all three

stream compartments. Possible links between the chemical quality of the individual compartments and the ecological stream quality were then explored by using multivariate statistical analyses.

The evaluation of the chemical quality in the three stream compartments revealed a substantial influence on both stream water and hyporheic zone from the diffuse metal sources (aluminum, barium, copper, lead, nickel, zinc) of both geogenic and anthropogenic origin in the catchment. The release of metals (particularly copper, nickel, zinc) was additionally enhanced by acidification of the noncalcareous aquifer. Moreover, the approach combining an evaluation of the contaminant mass discharge of the known anthropogenic point sources in the catchment together with the in-stream contaminant mass discharge showed to be an effective tool to both display their mutual importance and to reveal "new" sources. It further demonstrated the importance of contaminated sites as a potential noteworthy source to continuously impact the chemical stream quality (> ½ tonne per year of organic xenobiotics). An assessment of the chemical patterns (similarities) along the investigated stream corridor made it possible to link the chemical quality to a detected ecoresponse in the invertebrate communities.

This study thus demonstrated significant ecological impact resulting from the additional chemical stress of the inflow of a contaminated groundwater plume. The mechanism for this impact indicated that this was not caused solely by the presence of organic xenobiotics, but also by the strongly reduced redox conditions (e.g. high concentrations of dissolved iron and manganese) and secondary effects (e.g. high concentrations of dissolved arsenic), as a result of the organic degradation (e.g. benzene, toluene, ethylbenzene, and xylene) in the plume. The ecological impact was detected predominantly in the organisms living in the upper bed sediment. The sensitivity was especially pronounced in the meioinvertebrate community, which could be a promising tool to standardize the characterization of the ecological quality of streams considering their ubiquitous distribution throughout all ecoregions.

The methodology developed here, applying a holistic evaluation of both the chemical and ecological stream quality, thus demonstrates the importance for future risk assessments to include multiple compounds (i.e. organic and inorganic chemical stressors) and stream compartments to locate key sources and risk drivers. The approaches and findings in this thesis could truly be helpful for management and future remediation of mixed land use stream systems.

Dansk sammenfatning

Stigende befolkningstal og den industrielle udvikling har gennem det sidste århundrede medført store ændringer af landskabet på globalt niveau. Byudvikling og stigende landbrugsproduktion har forårsaget både hydromorfologiske ændringer og kemiske påvirkninger fra, diffuse forureningskilder (f.eks. geogenisk og landbrugsaktiviteter) og punktkilder (f.eks. spildevandsudledning og forurenede grunde). Ferskvandsmiljøerne såsom vandløb er særligt udsatte, da disse er tæt forbundet til de hydrologiske oplande via deres afdræning. Forringelsen af det naturlige landskab, skabt af de forskelligartede arealanvendelser, kan medføre kritiske ændringer og sætte vandløbets økosystem under pres.

Påvirkningen fra de forskellige foreningskilder medfører en blanding af diverse kemiske stoffer, som kan føre til en forringelse af den kemiske kvalitet i de forskellige dele af vandløbets elementer (åvandet, vandet i den hyporheiske zone og åbundssedimentet). Påvirkningen af de specifikke å-elementer afhænger yderligere af transportvejen for de kemiske stoffer, stoffernes fysisk-kemiske egenskaber, samt de hydrologiske og biogeokemiske forhold i og omkring vandløbet. Den resulterende påvirkning af de forskellige elementer varierer derfor både i tid og rum. På trods den stigende forståelse af denne kompleksitet er der stadig et væsentligt behov for at udvikle en helhedsorienteret tilgang til risikovurdering af de potentielle påvirkninger af vandløb.

For at forbedre forståelsen af sammenhængen mellem den kemiske kvalitet af vandløbets tre elementer og kvaliteten af vandløbets økosystem, blev der foretaget omfattende undersøgelser ved Grindsted Å, som blev anvendt som værkstedsområde i dette PhD projekt. Oplandet til det grundvandsfødte vandløb rummer både by og landbrugsarealer, samt to stærkt forurenede grunde. For at vurdere de væsentligste potentielle påvirkninger blev både organiske og uorganiske kemiske stoffer evalueret i de tre vandløbselementer. For at bedømme betydningen og størrelsesordenen af de forskellige påvirkningskilder blev en kombination af tre kendte metoder anvendt: forureningsflux, toksisk potentiale og tærskelværdier. For at kunne bedømme potentielle økologiske påvirkninger af ændringer i den kemiske kvalitet af de tre vandløbselementer blev både makro- og meioinvertebrat samfund evalueret, da de repræsenterer forskellige biologiske miljøer i vandløbet. Mulige sammenhæng mellem den kemiske kvalitet af de tre elementer og den økologiske kvalitet blev undersøgt ved hjælp af multivariat statistik.

Evalueringen af den kemiske kvalitet i de tre vandløbs-elementer viste, at både åvandet og den hyporheiske zone var stærkt påvirket af både diffuse geogeniske og antropogene kilder til metaller (aluminium, barium, kobber, bly, nikkel, zink). Frigivelsen af metallerne (kobber, nikkel, zink) blev yderligere forstærket af en forsuring af den kalkfattige undergrund. Den kombinerede evaluering af forureningsfluxen fra de kendte punktkilder (forurenede grunde, spildevandstilledninger) i oplandet og de estimerede forureningsfluxe viste sig at være effektivt til at vise kildernes indbyrdes betydning for vandløbet. Der blev også afdækket ”nye” forureningskilder på baggrund af forureningskoncentrationen i åvandet og vandføringen. Det viste sig yderligere, at forurenede grunde med stærkt forurenede grundvandsfaner er en betydelig kilde til en kontinuert påvirkning af vandløbskvalitet (>½ ton per år af organiske miljøfremmede stoffer udsiver til Grindsted Å).

Ved at afdække kemiske mønstre ned igennem vandløbet var det muligt at forbinde kemisk kvalitet med signifikante ændringer i de økologiske samfund. Studiet viste yderligere, at indsivningen af den stærkt forurenede grundvandsfane gav en signifikant nedgang i den økologiske kvalitet. Denne påvirkning skyldes ikke alene tilstedeværelsen af de organiske miljøfremmede stoffer, men også de stærkt reducerede forhold og de sekundære effekter (f.eks. høje koncentrationer af opløst jern, mangan og arsen) i den hyporheiske zone. Forekomsten af disse sekundære effekter er et resultat af nedbrydningen af de miljøfremmede organiske stoffer i forureningsfanen. Den økologiske forringelse var især bemærkelsesværdig for de organismer, som levede i selve åbunden. Især meioinvertebrate samfundet viste stor følsomhed. Denne følsomhed kunne på baggrund af deres uniforme tilstedeværelse på tværs af ferskvandsøkoregioner være et lovende redskab til at standardisere karakteriseringen af den økologiske kvalitet i vandløb.

Anvendelsen af en helhedsorienteret evaluering af den kemiske og økologiske kvalitet viste vigtigheden af at medtage både organiske og uorganiske kemiske stoffer i de forskellige vandløbselementer. Det åbnede mulighed for at bestemme/lokalisere hovedkilderne og risikoaktørerne i oplandet påvirket af både byområder og landbrug. Denne metodiske tilgang, som er udviklet, kan derfor være meget nyttig i forvaltningen af områder med ”blandet” arealanvendelse, samt planlægning af fremtidige indgreb og oprensninger af forureningskilder.

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Abbreviations

ANOVA	ANalysis Of VAriance
BS	Bed Sediment
BTEX,N	Benzene, Toluene, Ethylbenzene, m-/p- and o-Xylenes, Naphthalene
CAHs	Chlorinated solvents
CMD	Contaminant Mass Discharge
DHQI	Danish Habitat Quality Index
DSFI	Danish Stream Fauna Index
EC50	Effective Concentration where 50% of a population exhibit a response
EPT	Ephemeroptera, Plecoptera and Tricoptera taxa
EQS	Environmental Quality Standard
GDD	Groundwater Daughter Directive
HCA	Hierarchical Cluster Analysis
HZ	Hyporheic Zone
IC50	Immobilising Concentration causing a 50 % response
LC50	Lethal Concentration causing 50% mortality
nMDS	Non-metric MultiDimensional Scaling
PCA	Principal Component Analysis
PNEC	Predicted No Effect Concentration
PW	PoreWater
SPEAR	SPEcies At Risk
SS	Suspended Sediment
SW	Stream Water
TU	Toxic Unit
WFD	Water Framework Directive

1 Introduction

1.1 Background and motivation

Since the 1950s the human population has grown exponentially (WWF, 2016) leading to land use changes of significance on a global scale. The alterations created by the increased activities such as urban and agricultural expansion, however, have caused a wide range of pressures and critical threats to the health of ecosystems for example those of surface water bodies (Yu et al., 2014; Vörösmarty et al., 2010).

The impacts of the resulting multiple stressors are especially pronounced in freshwater systems, such as streams, as they are highly connected to the surrounding area through their draining system (Fausch et al., 2010). Impairment is produced from both chemical sources as well as physical and hydrological alterations of the watercourse (Carpenter et al., 2011).

The importance of addressing this pressing environmental issue/problem was further stressed by the enactment of the European Water Framework Directive (WFD) in 2000 and the corresponding Groundwater Daughter Directive (GDD) in 2006, which required all member states to ensure good chemical and ecological status of both groundwater and surface waters by the end of 2027 (European Commission, 2017). Moreover, this has encouraged the extension of the 2014 Danish soil act to include contaminants that poses a risk to surface water as well as groundwater. This has enforced contaminated sites to be included in the risk assessment of surface water bodies.

The need for understanding the interaction between surface water and groundwater has thus attained a renewed importance, and a framework is required to diagnose the primary threats to stream quality. According to the WFD, the chemical status of surface water bodies is defined by a set of environmental quality standards (EQS) on priority substances (EQS Directive (2008/105/EC) in (fresh and marine) water concentrations. A standard value is used that is based on the lowest short-term lethal concentration (50 %, LC50) of algae, crustaceans and fish multiplied by a safety factor, typically 10,000 (EU, 2011). Each member state can, however, have EQS values for additional compounds, as enacted by the Danish EPA (2010, BEK nr. 1022). The relationships between the quality elements (biological, hydromorphological and physico-chemical) in the classification of the ecological status is defined in a more holistic manner leaving the Member States free to define the details of their own assessment tool (European Commission, 2017).

The chemical sources in these mixed land use stream systems comprise both (i) diffuse pollution of the stream through e.g. surface run-off, tile drains or groundwater-stream water interaction; and (ii) point effluence from e.g. wastewater outlets and polluted groundwater discharge from contaminated sites (McKnight et al., 2012). Once in the stream system, contaminant distribution is dependent on factors, such as physico-chemical properties, hydrological processes, and redox conditions, that lead to temporal and spatial variation in the stream compartments, e.g. stream water (SW), hyporheic zone (HZ), or bed sediments (BS).

The growing understanding of this complexity and the high probability of multiple stressors, including mixtures co-existing and interacting in these stream systems (Heys et al., 2016; McKnight et al., 2012; Sánchez-Montoya et al., 2010), have encouraged a shift to a shift toward holistic risk evaluation. However, collecting representative field data for a chemical risk assessment in a mixed land use stream system where both temporal and spatial variations occur is complicated. Field-based studies have approached this by focusing on one chemical compound group in one compartment (e.g. SW) throughout a catchment (pesticides: Wittmer et al., 2010; metals: Yu et al., 2014; general water chemistry: Ding et al., 2016) or several compound groups (Malaj et al., 2014; Sabater et al., 2016). These approaches were accompanied by localization of the main sources based on the knowledge of the land use or particular sources (e.g. wastewater outlets) in the catchments. However, the growing acceptance of the complex distribution of the chemical stressors have encouraged more recent field studies to include multiple stream compartments typically encompassing SW, BS (e.g. Fairbairn et al., 2016) and additionally suspended sediment (SS) (Nazeer et al., 2014) in the chemical risk assessment. However, the potential impact of groundwater plumes from contaminated sites to the chemical and ecological quality in mixed land use stream systems is still inadequate in risk assessments (Roy and Bickerton, 2012).

1.2 Research objectives and aims

The main objective of this PhD thesis was to investigate and improve the understanding of the presence of multiple chemical stressors and the ecological stream health in a mixed land use stream system. The specific aims were thus to:

- Provide an overview of existing risk assessment approaches based on field studies in mixed land use stream systems
- Evaluate the potential for substantial groundwater plumes from contaminated site to impact ecological stream quality
- Develop a methodology for assessing risk in mixed land use stream systems to determine the main chemical stressors detected in the stream corridor and identify key sources and risk drivers
- Investigate possible links between chemical and ecological stream quality using the methodology generated for chemical stressors in these systems

1.3 Structure

In Chapter 2, the importance of risk assessment of mixed land use stream systems are reviewed. An overview of current approaches and their limitations to predict ecological hazard in the stream systems is described. In Chapter 3, an overview of the study site and the principles behind the chosen approaches for a holistic stream assessment are explained. Next, the methodologies applied are provided. The assessment and link between the chemical and the ecological quality throughout the investigated stream corridor is outlined in Chapter 4. The results and their significance are discussed and placed in context with the existing literature in Chapter 5. Conclusions and perspectives are given in Chapters 6 and 7.

2 Studies of mixed land use stream systems

The degradation of both chemical and hydromorphologial quality in mixed land use stream systems creates critical changes that can dramatically alter a system's ecosystems and biodiversity (Schäfer et al., 2012; Malaj et al., 2014; Haase et al., 2013; Vörösmarty et al., 2010). The resulting diverse chemical stressors reach the streams through different pathways, e.g. groundwater-stream water interaction, surface run-off, wastewater outlets or tile drains, as illustrated in Figure 1. This contamination dynamics of both temporal and spatial variations thus creates a complex picture where the distribution of contaminants in the stream system depends on a number of factors (e.g. physico-chemical properties, redox conditions, hydrological processes) that lead to diverse impacts within different compartments, e.g. SW, HZ, BS or suspended sediment (SS).

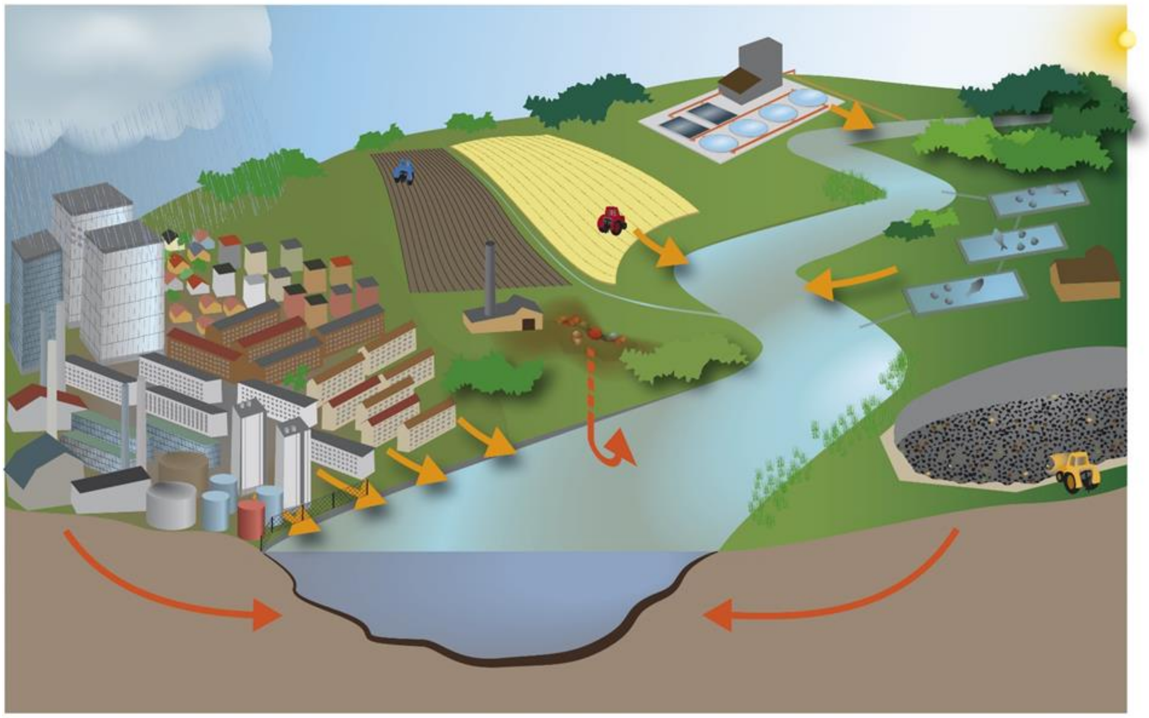

Figure 1. Conceptual sketch for a mixed land use stream system impacted by multiple sources from both urban (e.g. wastewater outlets, contaminated sites) and agricultural activities (e.g. crop production, fishfarms). The pathways of the chemical stressors to the stream are shown with orange arrows (e.g. groundwater-stream water interaction, surface run-off, wastewater outlets and tile drains). Source: Modified from Sonne et al. (**II**).

Within the last two decades, the growing understanding of the complexity in mixed land use stream systems (Stutter et al., 2007; Ding et al., 2016; Palmer et al., 2010) has shifted risk management toward a catchment scale focus (Table 1), as opposed to an isolated issue and/or contamination event focus (Barringer et al., 2010; Conant et al., 2004).

Table 1 provides an overview of risk assessment approaches conducted in mixed land use stream systems. This demonstrates that several field investigations have approached the chemical assessment by focusing on e.g. one compound group in one stream compartment (SW: Wittmer et al., 2010; Yu et al., 2014; Ding et al., 2016) or multiple compound groups (SW: Malaj et al., 2014; Kuzmanovic et al., 2016) in one stream compartment to locate the main sources. The latter investigations additionally linked the chemical assessment to toxicological (laboratorial estimates) and ecological (field observations) descriptors to examine potential key compounds that impact ecological quality. However, analysis of the chemical link was not conducted by using the detected concentrations (C_i) in the environment as in Berger et al. (2016). Instead, potential toxicity was determined by performing a threshold selection analysis with the LC50 for each chemical or by calculating the predicted toxicity of each detected compound with the toxic unit approach ($TU = C_i / LC50_i$; Tomlin, 2001).

The potential toxicity of the detected concentrations was yet in other studies evaluated and ranked by using established freshwater threshold values: EQS and the Predicted No Effect Concentration (PNEC) (Berger et al., 2016) or by recommended national criteria as in Stutter et al. (2007) using national Scottish threshold values.

In Castro-Catalá et al. (2016) the predicted toxicity was combined with a laboratory estimation of the overall site-specific toxicity of the investigated compartment, BS, by using both acute porewater and long-term whole-sediment exposure bioassays. The estimated toxicological descriptors were then statistically correlated with observed structural changes in one community, e.g. macroinvertebrates (SW: Kuzmanovic et al., 2016; BS: Castro-Catalá et al., 2016) or meioinvertebrates (BS: Höss et al., 2011), or several communities e.g. diatoms, macroinvertebrates, and fish (SW: Malaj et al., 2014) in the stream system.

A comprehensive number of ecological descriptors have been employed in the field-based studies (Table 1). These cover a large variety of analyses of the communities' compositions (e.g. trait specific; Stutter et al., 2007), species or genus level classification (e.g. taxon-specific changes; Berger et al., 2016) and calculations of specific sensitive ecological indices.

Conventional metrics are most sensitive to eutrophication and organic pollution in the SW compartment. These metrics include the Average Score Per Taxon (ASPT), Danish Stream Fauna Index (DSFI, a Danish version of ASPT), Species At Risk (e.g. $SPEAR_{organic}$ and $SPEAR_{pesticides}$) based on the benthic macroinvertebrate community as employed in Rasmussen et al. (2013; DSFI, $SPEAR_{pesticides}$) and Kuzmanovic et al. (2016; $SPEAR_{organic}$, $SPEAR_{pesticides}$). Less used indices in the literature are e.g. based on the benthic nematode community. A community that is exclusively embedded in the upper streambed compared to the macroinvertebrate community, which represents a broader habitat from 5-10 cm above to 5 cm below the streambed (Figure 2A). The nematode community was demonstrated to respond to the chemical quality of the BS in Höss et al. (2011). Based on nematode taxa, two indices, one sensitive to organic xenobiotic ($NemaSPEAR[\%]_{organic}$) and the other to metal ($NemaSPEAR[\%]_{metal}$) contamination in the BS were classified (Höss et al, 2011).

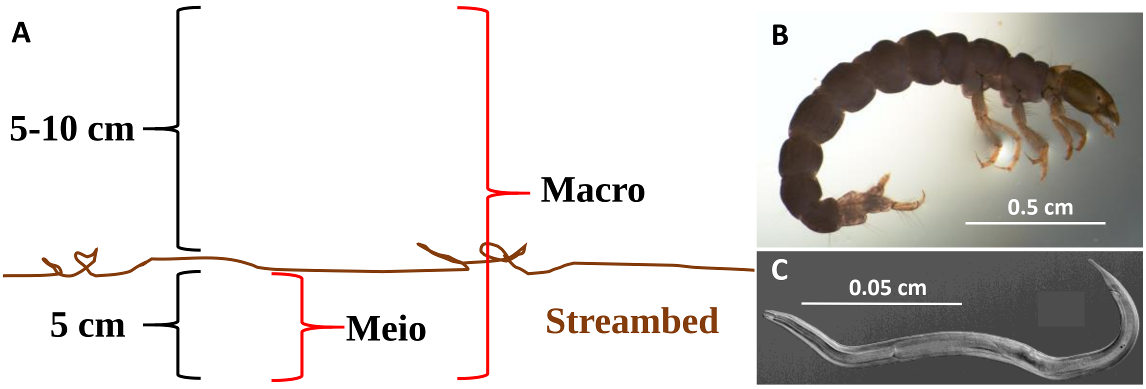

Figure 2: (A) The habitat of the benthic macroinvertebrate (Macro) and the meioinvertebrate (Meio) communities. A caddis fly larva (B) and nematode (C) are shown to illustrate the size difference in the macro- and meioinvertebrate communities, respectively. The invertebrate pictures (B-C) are courtesy of Prof. Dr. Walter Traunspurger, University of Bielefeld.

A few studies have evaluated multiple compartments for one compound group (SW, BS: Moon et al., 1994; SW, SS, BS: Stutter et al., 2007) or multiple groups (SW, SS: Rasmussen et al., 2013; SW, SS, BS: Nazeer et al., 2014; SW, BS: Fairbairn et al., 2015) in order to obtain a holistic picture of the chemical quality.

The physical properties of the stream system, e.g. sediment characteristics, have also been included in two BS studies (Castro-Catalá et al., 2016; Höss et al., 2011) to clarify if the changes in the benthic communities were governed by the grain size rather than chemical quality. Moreover, land use data has been used as an integrated factor with different, related stressors. For example, Berger et al. (2016) used land use data to correlate observed ecological changes with poor habitat quality and eutrophication. In Höss et al. (2011) the characterization of the hydro-morphological stream type (e.g. channel, barrage) was used as indirect measurements of the physico-chemical properties and chemical contamination. However, data from more detailed hydromorphology characterizations of each sampling station were only included in a few studies, as in e.g. Sabater et al. (2016) and Rasmussen et al. (2013) in the assessment of deviations in a community.

The investigations have covered a wide range of chemical stressors; however, some sources have received less attention in the field-based studies of mixed land use stream systems, such as the potential impact of contaminated sites through groundwater–stream water interaction (exceptions include Roy & Bickerton, 2012; McKnight et al., 2010). This is in part a result of a traditional subdivision of groundwater and surface water in risk assessment. Yet with the enactment of the WFD and the corresponding GDD, the need for understanding the interaction between groundwater and surface water and influence to water quality has consequently attained a renewed importance. Contaminated sites are moreover potential sources of a large variety of contaminants including chlorinated solvents, gasoline constituents, pharmaceuticals, inorganic macro-components and trace metals (McKnight et al., 2012; Roy & Bickerton, 2012; Christensen et al., 2001).

Thus the main focus of the literature today has been on SW, BS and SS in risk assessments (Table 1), however by excluding the assessment of the chemical quality in the HZ, a potential hazard to an ecosystem could be overlooked and its ecological quality misinterpreted. This further stresses the necessity of finding a suitable ecological community that represents the stream compartment habitat of risk to assess the ecological effect of the chemical quality.

Table 1: Literature review indicating state-of-the-art with respect to the risk assessment of mixed land use stream systems. The overview displays how each field-based study has approached this issue, including which stream compartment(s) and compound group(s) was explored, was the ecological quality in the stream assessed and by which organism(s), was the toxic potential of each detected compound estimated and by what method(s), were the chemical assessments linked to a source(s) and/or to ecological and toxicological descriptors and by what method(s), and which sources were investigated and what were the findings. Note the abbreviations stream compartment (COMP), contaminant compound group (CONT GR), stream water (SW), water from the hyporheic zone (HZ), suspended sediment (SS), suspended particulate matter (SPM), bed sediment (BS) and the two benthic communities: macroinvertebrates (macro) and meioinvertebrates (meio). Dashes indicate that the issue was not investigated.

Reference	COMP	CONT GR	Ecological quality	Toxic potential	Factor relationship	Investigated sources	Findings
One compound group in one stream compartment							
Wittmer et al. (2010)	SW	Pesticides	-	-	Time cross-correlated conc. with location and events	Identified associated point sources (wastewater discharge from urban drainage system), diffuse agricultural sources	Equally important sources
Yu et al. (2014)	SW, rain, storm water	Metals	-	-	Multivariate statistical analyses: (long-term investigations) conc. vs. land use, events	Impact of diffuse land use	Metals: Farmland constant source, urban runoff periodic source (vehicle traffic)
Ding et al. (2016)	SW	General water chemistry	Algae growth (Chl-α, eutrophication indicator)	-	Multivariate statistical analyses and model: conc. vs. Chl-α, land use and geomorphic regions (scale-effect)	Impact of diffuse land use	Poor water quality: cropland, orchards, grassland in mountain catchments and urban land use in plain catchments Best estimate of the variation: land use on a catchment scale
Multiple compound groups							
Höss et al. (2011)	BS	Organic xenobiotics (e.g. pesticides, polyaromatic hydrocarbons), metals	Meio (nematodes) community	TU(PW) (macro)	Multivariate statistical analyses: ecological and toxicological descriptors, BS properties	Not source oriented	Development of two nematode indices (meioinvertebrate) sensitive to metal and organic xenobiotic BS contamination: NemaSPEAR (organic, metals)
Malaj et al. (2014)	SW	Organic xenobiotics (e.g. pesticides, brominated flame retardants)	Diatoms, macro and fish communities	Acute(Cmax/10) & chronic (1/1000, 1/100, 1/50) threshold selection using LC_{50} values (algae, macro, fish)	Multivariate statistical analyses: land use, ecological and toxicological descriptors	Impact from diffuse land use (natural vegetation, agricultural & urban practices)	Organic chemicals are an environmental problem on a continental scale

Sabater et al. (2016)	SW	Organic xenobiotics (e.g. herbicides, antibiotics, hormones)	Diatoms (in the biofilm), invertebrate community	-	Multivariate statistical analyses: ecological descriptors vs. land use, conc., BS properties, hydrological characteristics	Impact of diffuse land use	Ecological degradation from increasing agricultural and urban-industrial activities, high water conductivity, dissolved organic carbon and inorganic N and high conc. pharmaceutical & industrial compounds
Kuzmanovic et al. (2016)	SW	Organic xenobiotics (e.g. pesticides, pharmaceuticals), metals	Macro community $SPEAR_{organic}$ $SPEAR_{pesticides}$	TU(SW) (algae, macro, fish)	Multivariate statistical analyses: land use, ecological and toxicological descriptors	Not source oriented	SW: Metals posed acute risk at 44% of the sites, organic chemicals (mainly pesticides) 42%. Several emerging contaminants pose chronic effects risk
Castro-Catalá et al. (2016)	BS	Organic xenobiotics (e.g. endocrine disrupting compounds, pharmaceutical active compounds), metals	Macro community	TU(PW)(algae, macro), acute porewater & whole-sediment exposure tests	Multivariate statistical analyses: ecological and toxicological descriptors, toxicity tests, BS properties	Not source oriented	Organophosphate insecticides and metals main stressors to BS toxicity
Berger et al. (2016)	SW	Organic xenobiotics (e.g. pesticides, pharmaceuticals, plasticisers, flame retardants)	Macro community (over time) using	Threshold Indicator Taxa Analysis (TITAN), EQS, PNEC	Multivariate statistical analyses: change points in taxon and conc., conc. vs. land use, catchment size	Impact of diffuse land use, source associated compounds	WWTP: Strong effects to wastewater-associated compounds. Observed ecological effects at conc.<EQS, PNECs at change points
Multiple stream compartments							
Moon et al. (1994)	SW, BS	Metals (Cu, Pb, Zn)	-	-	Time cross-correlated conc. (SW and BS), land use, SB properties	Impact from diffuse land use, identified associated point sources (domestic effluents)	Cu, Pb, Zn domestic effluents (urban) >>rural land use during dry periods
Stutter et al. (2007)	SW, SPM, BS	General water chemistry, P, N, C	Algae growth (Chl-α), macro community,	Recommended threshold criteria in SW (Scottish EPA)	Multivariate statistical analyses: conc. (SS, BS),	Diffuse land use sources	Biologically available P: Greatest pressure from agricultural land use was seen in SPM > BS (in organic C).

			trait-specific changes		land use, catchment size, ecological descriptors, physical stream parameters		Chl-α increase correlated with increase in P-contaminated SPM
Multiple compound groups and stream compartments							
Rasmussen et al. (2013)*	SW, SS	General water chemistry, Organic xenobiotics (e.g. pesticides, CAHs, petroleum hydrocarbons)	Macro community DSFI, $SPEAR_{pesticides}$	TU(SW, SS) (macro)	Multivariate statistical analyses: ecological and toxicological descriptors, physical stream parameters	Diffuse land use, contaminated sites, low base-flow due to water abstraction, hydromorphological quality	Identified numerous chemical and hydromorphological impacts. Not able to rank the sources based on the major ecological impairments. $SPEAR_{pesticides}$ indicates insecticides were an essential contributor
Nazeer et al. (2014)	SW, SS, BS	Heavy metals, general water chemistry, nutrients	Bacteria	Water quality index (WQI) based on presence bacteria, metals, nutrients, pesticides	Time cross-correlated conc. (SW, SS, BS), WQI, with location and events	Not source oriented	SW: Nutrient load high during pre-monsoon season, metals high during post-monsoon. Metals: SS>BS from both natural processes and anthropogenic activities. Cd, Zn, Pb threats to aquatic ecosystems
Fairbairn et al. (2015)	SW, BS	Organic xenobiotics (e.g. personal care products, pesticides, human and veterinary medications)	-	-	Time cross-correlated conc. (SW, BS) with location and events	Diffuse land use	Spatial and temporal analysis: pharmaceuticals & personal care products highest in SW+BS with population density (>100 people/km^2) and %developed land use (>8% of the sub-watershed area). Pesticides in agricultural land use. Measured more in BS than predicted. Seasonal in SW not in BS

3 Site description, approach and methodology

3.1 Study site

The investigation was conducted along 16 km of a typical Danish lowland stream in the Western part of Denmark, where both agricultural and urban activities were represented (Figure 3). The study site, Grindsted stream, is naturally meandering with very few stream modifications and landscape changes since 1945 (confirmed by aerial photos, for examples see Appendix Figure S1). The geology is characterized by a calcareous deficient Quaternary sand formation (10-15 mbgs) underlain by Tertiary sand formations (Barlebo et al., 1998; Heron et al., 1998).

The major potential contaminant sources in the catchment impacting the stream system (Figure 3) were identified to include agricultural activities (comprising 54 % of the catchment area land-use), a wastewater treatment plant (WWTP), two fish farms, and urban sources from Grindsted town (12 % of the catchment area). The urban sources include stormwater runoff, traffic, and biocide applications.

There are two large contaminated sites located within 2 km of the stream: a pharmaceutical factory site and an abandoned, unlined municipal landfill (Kjeldsen et al., 1998a-b). The two sites are contaminated by multiple organic xenobiotics including pharmaceuticals (e.g. sulfonamides, barbiturates) and volatile organic compounds (e.g. benzene, toluene, ethylbenzene, m-/p- and o-xylenes, naphthalene, i.e. BTEX,N) (NIRAS, 2009; COWI, 2011; Kjeldsen et al., 1998a; Holm et al., 1995; Rügge et al, 1995). The groundwater plume from the factory site also contains high concentrations of lithium; bromide; and the chlorinated ethenes tetrachloroethylene (PCE), trichloroethylene (TCE), cis-1,2-dichloroethylene (cis-DCE), and vinyl chloride (VC).

The contaminated groundwater plume has been outlined from the factory site, and the flow is toward the stream (COWI, 2011; Balbarini et al, 2017). The main discharge zones of the contaminated factory plume were located in Grindsted stream in 2012 in the area of stations 4, 6 and 8 (Figure 1) by Nielsen et al. (2014).

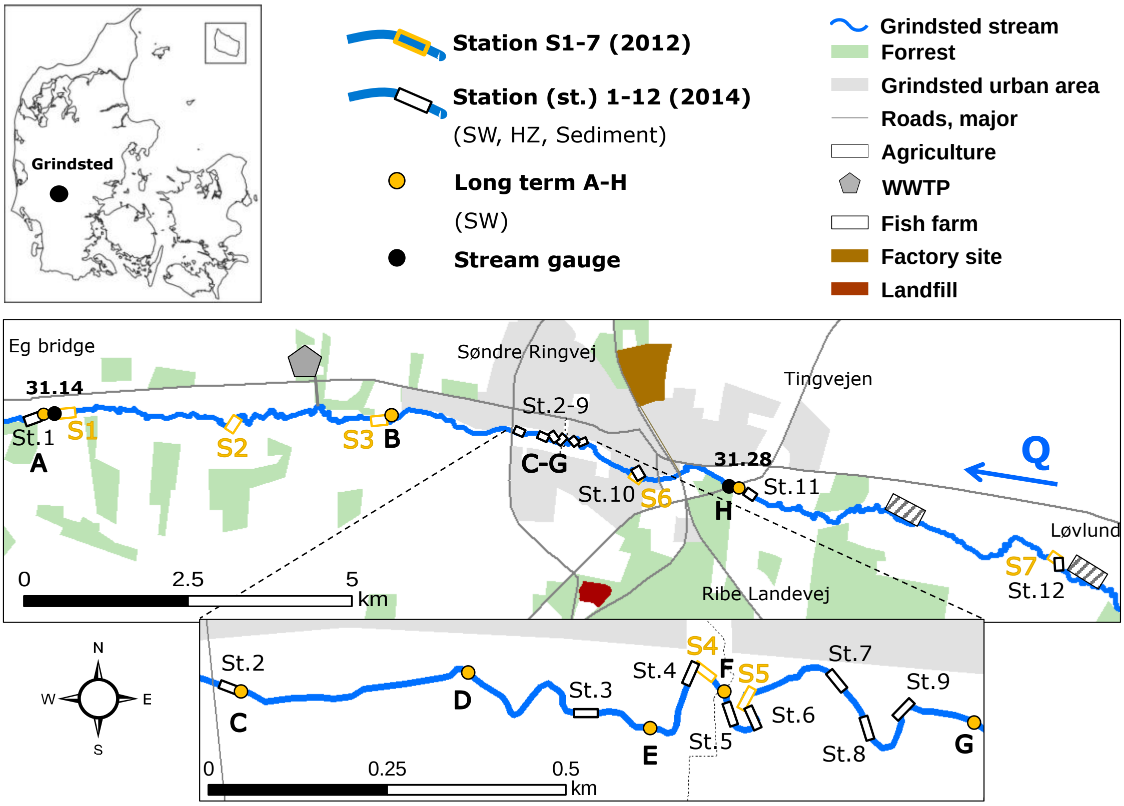

Figure 3: Land use distribution along the investigated stream corridor. The locations of the factory and landfill sites are illustrated with a brown and a red polygon, respectively. The WWTP location is shown with a grey pentagon and the two fish farms with striped rectangles. The stream flow direction, Q, is shown with a blue arrow. The positions of the two stream gauges, 31.28 (Tingvejen) and 31.14 (Eg Bridge), are shown with black dots. The placement of the sampling stations investigated are shown as open rectangles (orange in August 2012 and black in April 2014) and the eight long-term stations, monitored from August 2012 to March 2015 with yellow dots (labeled A-H). Modified figure from Sonne et al. (**II**).

3.2 Approach for stream assessment

First we investigated the chemical and ecological quality along a discharging contaminant plume gradient to assess the importance of the groundwater pathway (Nielsen et al., 2014; Rasmussen et al., **I**). For details of the study site, see Section 3.2. The chemical assessment included organic xenobiotics monitored in both the SW and the HZ, as a proxy for groundwater contamination) (Section 3.3). At each sampling station, the general water chemistry in the SW was assessed and the hydromorphology of the stream was characterized in order to establish if the stations had comparable properties.

The ecological quality was evaluated by characterizing the benthic macroinvertebrate communities (Section 3.4). Special attention was given to trait and taxon specific changes as well as to morphological deformities (e.g. mentum, antennae) of the Chironemidae larvae (Diptera). Diptera is a family embedded in the BS/HZ that has been shown to develop abnormalities as a response to anthropogenic and environmental stress (Lenat, 1993; Al-Shami et al., 2010). Moreover, the ecological macroinvertebrate index sensitive to organic xenobiotic SW contamination ($SPEAR_{organic}$) was evaluated and compared to currently used indices sensitive to eutrophication (ASPT, DSFI) and pesticide contamination in SW ($SPEAR_{pesticides}$).

Secondly, we investigated the same mixed land use stream system (Section 3.2), but particular attention was given to previously measured compounds (Sonne et al., II) in order to offer a holistic picture of the chemical quality and to identify and rank all relevant sources. To assess and link probable chemical impacts to their respective sources in the catchment, samples were taken from all three stream compartments: SW, HZ and BS (section 3.3). To cover the potential chemical stressors, the chemical assessment included analysis of the general water chemistry, trace metals and organic xenobiotics (e.g. chlorinated solvents, pharmaceuticals, pesticides and metabolites). To evaluate the organic and inorganic chemical stressors in the stream corridor, we combined three approaches: (i) in-stream contaminant mass discharge (CMD, Section 3.6), (ii) Toxic Units (Section 3.7) and (iii) threshold values (e.g. EQS (SW), sediment (US EPA, 2006) (Section 3.8), general water chemistry (DCE, 2012)) to be able to quantify and equally assess the chemical quality in the three stream compartments.

The principle behind the in-stream CMD approach is to estimate the mass of contaminant per unit time in the stream simply by measuring the SW concentration (mass/volume) and stream flow (volume/time) at the point of fully mixed conditions downstream of a discharge source (Troldborg et al., 2012). By using this method, it is possible to quantify anthropogenic point sources and evaluate their magnitude and importance in a stream system (Aisopou et al., 2015; Rønde et al., 2017). Assessment with the Toxic Units approach investigates the toxic potential of organic xenobiotics and metals in the SW, HZ, and pore water (PW) of the bed sediment. By comparing the detected compounds with representative threshold values (international or national, Section 3.7) to maintain a healthy stream it is possible to make a conservative evaluation/screening of both the general stream water quality in the SW and the chemical quality of the BS in the stream corridor. Concentrations can further be compared to typical national values in order to determine if significant impairment has occurred.

Thirdly we grouped the stream sections according to their contaminants, ecotoxicity and biogeochemical similarities in the SW, HZ and BS using multivariate statistical analyses (Section 3.9). In order to investigate possible links between the chemical quality of each stream compartment with statistically significant structural changes in the benthic macro- and meioinvertebrate community compositions (Sonne et al., **III**). The two communities were chosen because they represent different habitats in the stream system, as shown in Figure 2, and thereby provide a broader coverage of potential impacts in the stream ecosystem due to the chemical quality of the three stream compartments (SW, HZ, BS). A detailed characterization of the hydromorphological quality of the stream (Section 3.5) was also assessed at each of the sampling stations and correlated to the observed changes in the communities. Furthermore, the dominant streambed sediment type (i.e. gravel, sand, organic matter) was statistically correlated with its respective macroinvertebrate subsamples to elucidate if significant changes were substrate dependent.

3.3 Water and sediment sampling

To investigate the chemical quality and link chemical impacts to their respective sources in the catchment, samples were taken from the SW, HZ at 40-60 cm depth, and the sediment from the upper 5 cm of the BS (Figure 4, Figure 6). For a detailed description of the sampling methods, see material and methods in Rasmussen et al. (**I**) and Sonne at al. (**II**). The samples were collected over the period May 2012 to March 2015.

In 2014, a special sampling design was created to conduct simultaneous chemical and ecological assessment at each sampling location in Grindsted stream. The chemical assessment was conducted on three stream compartments: SW, HZ and BS, as shown in Figure 5. Figure 5A illustrates the sampling stations in April 2014 and Figure 5B in August 2014.

Eight stream water sampling points (A-H, Figure 3) were furthermore revisited/sampled seven times in the period August 2012 to March 2015 (August 2012; April, May, June, August and October 2014; and March 2015) to monitor the temporal variation of the chemical quality of stream water and the in-stream fate of the organic xenobiotic compounds (Sonne et al., **II**; Rønde et al., 2017). Cross sectional SW sampling was conducted in 2014 and 2015 to determine the mixing of contaminants in the stream downstream of the main discharge zone of the factory site contaminant plume (Rønde et al., 2017).

The water samples were analyzed for general water chemistry, selected trace metals and organic xenobiotics, i.e. CAHs, BTEXN, pesticides and associated metabolites, and pharmaceutical compounds. The streambed sediment was analyzed for grain size distribution, total organic carbon (TOC), selected trace metals and hydrophobic pesticides and metabolites (**II**, Appendix Table S1). For a detailed overview and chemical analysis, see Sonne et al. (**II)**.

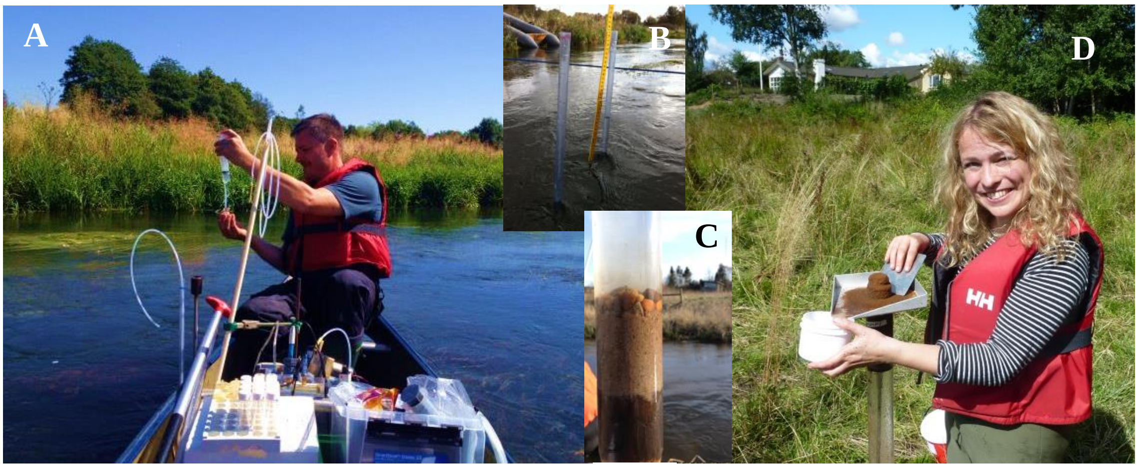

Figure 4: (A-B) The water sampling was mainly conducted from a canoe during the campaigns and (C) the sediment sampling in August 2014 due to high water level in the stream. (D) A device developed in-house by Department of Bioscience (AU) to sample the upper streambed sediment (5 cm) from the sediment core.

3.4 Sampling and characterization of the invertebrate communities

In August 2012, macroinvertebrates were sampled strategically at seven locations along the stream (S1-S7; Figure 3) to investigate if the discharge of the contaminated groundwater plume had an ecological impact. At each location twelve subsamples were taken using a 500-cm^2 surber sampler (Figure 6A) and collected along a 50 m stream stretch as shown in Figure 5A. The characterization of the community and the identification of morphological deformities of the chironomidae larvae were based on a pooled sample from each location (Rasmussen at al., **I**).

In April 2014, macroinvertebrates were sampled at twelve sampling stations (1-12; Figure 3) tactically placed to investigate/assess potential ecological impacts to the chemical quality of the SW, HZ and BS along a mixed land use catchment. The macroinvertebrates were again sampled along a 50 m stream stretch (Figure 5A and 8A); however the twelve subsamples at each station were characterized individually. In August 2014, meioinvertebrates were sampled in triplicate using kayak coring at stations 2, 4, 6, 8 and 10 (Figure 3, Figure 5 and Figure 5B). Note each station in August 2014 represents a 5 x 5 m^2 area. The community of each subsample was characterized individually. For details, see Sonne et al. (**III**).

All invertebrates were identified to the lowest possible taxonomic level. The number of species ($1x1m^2$), total abundance, species richness [Margalef's richness: $D_{Marg} = (S-1)/Ln(N)$], Evenness [Pielou's Evenness: $J' = H'/Ln(S)$] and Shannon's Diversity Index [$H' = \Sigma(p_i*Ln(p_i)$, p_i = fraction of species i)] were calculated for both communities. For details see Sonne et al. (**III**).

In 2012, the score of the macroinvertebrate indices: $SPEAR_{organic}$, $SPEAR_{pesticides}$ (Beketov and Liess, 2008; Liess and von der Ohe, 2005), ASPT (Armitage et al., 1983) and DSFI (Skriver et al., 2000) were calculated based on the community at each sampling station (Rasmussen et al., **I**) .

In August 2014, a nematode index sensitive to chemical stress, $NemaSPEAR_{genus}$[%]-index, was calculated according to Höss et al. (2017). The two NemaSPEAR indices ($NemaSPEAR[\%]_{organic}$, $NemaSPEAR[\%]_{metal}$) sensitive to metals and organic xenobiotic contamination in the BS were furthermore classified according to Höss et al. (2011).

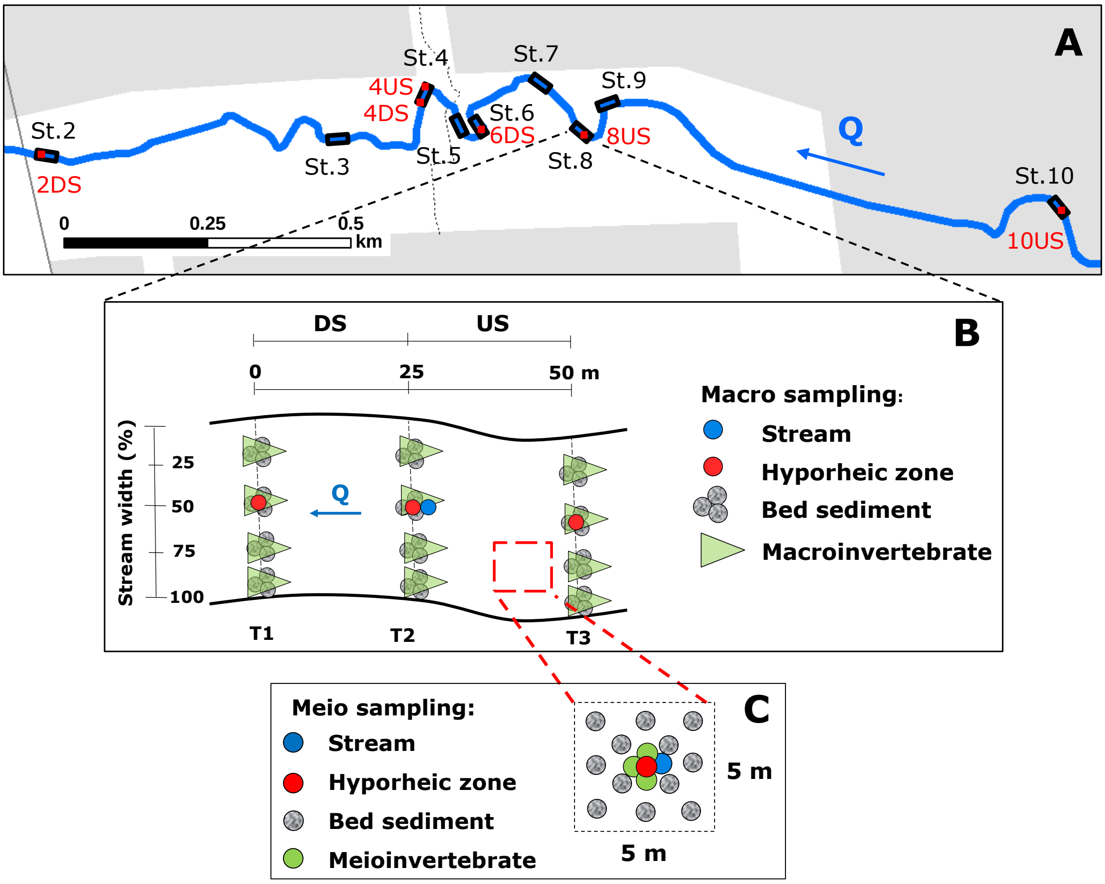

Figure 5: (A) Locations of sampling stations 2-10 in April 2014 are shown in black. The placement of the small sampling stations in August 2014 (2DS, 4DS, 4US, 6DS, 8US and 10US) are shown in red. (B) Design of a macroinvertebrate sampling station representing a 50 m stream stretch in April 2014. The distribution of macroinvertebrate sampling along each of the three transects (T1, T2, T3) is shown with green triangles. The chemical sampling location of the stream water is shown with a blue dot, the hyporheic zone at 40-60 cm depth with a red dot and the bed sediment with grey dots. The same is shown for the smaller meioinvertebrate sampling stations (C), where each represents a 5x5m^2 area of the stations (2, 4, 6, 8 and 10), that were re-investigated in August 2014. The location in the larger macroinvertebrate sampling stations was specified as US (upstream) if placed between T2 and T3; and DS (downstream) if it was placed between T1 and T2. Meioinvertebrate sampling is illustrated with green dots. Figure from Sonne et al. (**III**).

Figure 6. First, (A) macroinvertebrate sampling was conducted using a 500-cm^2 surber sampler along each of the three transects at 25%, 50%, 75% and 100% from the stream bank. Secondly, (B) streambed sediment was sampled along each of the three transects by taking kayak cores in triplicate (Kronvang et al., 2003). (C) Demonstration of the sampling along the transects at station 3 in April 2014. For the detailed sampling strategy, see Figure 5.

3.5 Stream characteristics and hydromorphological quality

The seasonal variation of the stream was identified by monitoring of the stream water level (h) and flow (Q) by two online gauges (for locations see Figure 3). In order to establish a relationship between Q and h, manual measurements of the stream level and flow were taken at both gauges (Sonne et al., **II**). Additional stream level and flow measurements were used to describe the local variation along the investigated stream stretch (Orbicon A/S 2012; Rasmussen et al., **I**).

The quality of the physical stream dimensions and habitat properties was assessed at each invertebrate sampling station in both 2012 and 2014 using the Danish Habitat Quality Index (DHQI, Pedersen et al., 2006). The index assesses the quality by evaluating 16 descriptors of sensitive habitat parameters, such as physical variation of the investigated stretch, submerged vegetation in the stream and coverage (%) of the dominant streambed sediment type (stones, gravel, sand, mud) (Rasmussen et al., **I**; Sonne et al., **III**). The final score can range from 12 to 63. The threshold level for good quality is 26 (Dunbar et al., 2010).

In order to investigate if significant changes in the macroinvertebrate communities were determined by the sediment type in April 2014. A minimum of three kicks were taken at each station for each habitats dominated by gravel, sand and organic matter. The small meioinvertebrate sampling stations were intentionally placed in areas of fine streambed sediment by a visual judgement to make sure the six stations represented the same habitat. This was later confirmed via grain size analysis, as described in Sonne et al. (**III**).

3.6 In-stream estimation of contaminant mass discharge

To quantify anthropogenic point sources and evaluate their magnitude and importance in the investigated stream corridor, the in-stream CMD were estimated as suggested in Aisopou et al. (2015). The approach is based on a simple mass balance and plug flow interpretation to describe the mixing and transport of a discharged compound in a stream (Rønde et al., 2017; Sonne et al., **II**). If it is assumed that attenuation processes have little or no importance for the stream water concentration and that no accumulation takes place within the stream segment of interest, the mass balance can be described by:

$$CMD_{bg} + CMD_{input} = CMD_{total} \quad (1)$$

where CMD_{bg} is the background mass discharge of a specific compound, CMD_{input} is the mass discharge of the compound and CMD_{total} is the total mass of the compound at the point of fully mixed conditions in the stream.

Assuming the mass of a compound entering the system is equal to the mass leaving the system, the output can be expressed as:

$$c_{bg} * Q_{bg} + c_{input} * Q_{input} = \left(Q_{bg} + Q_{input}\right) * c_{mix} \quad (2)$$

where c is the stream water concentration and Q the water discharge. Moreover, if the stream discharge at the point of fully mixed conditions, Q_{mix}, is unknown and the discharge of the input, Q_{input}, is relatively small compared to the discharge of the stream, Q_{bg}, Q_{input} can be neglected. Additionally, if c_{bg} is below the quantification limit, the mass discharge (Equation 2) can be rewritten as (Figure 7):

$$CMD = C_{mix} * Q \tag{3}$$

The investigation of Grindsted stream has shown that fully mixed conditions were achieved on average within 150 m downstream of a discharge zone (Rønde et al., 2017).

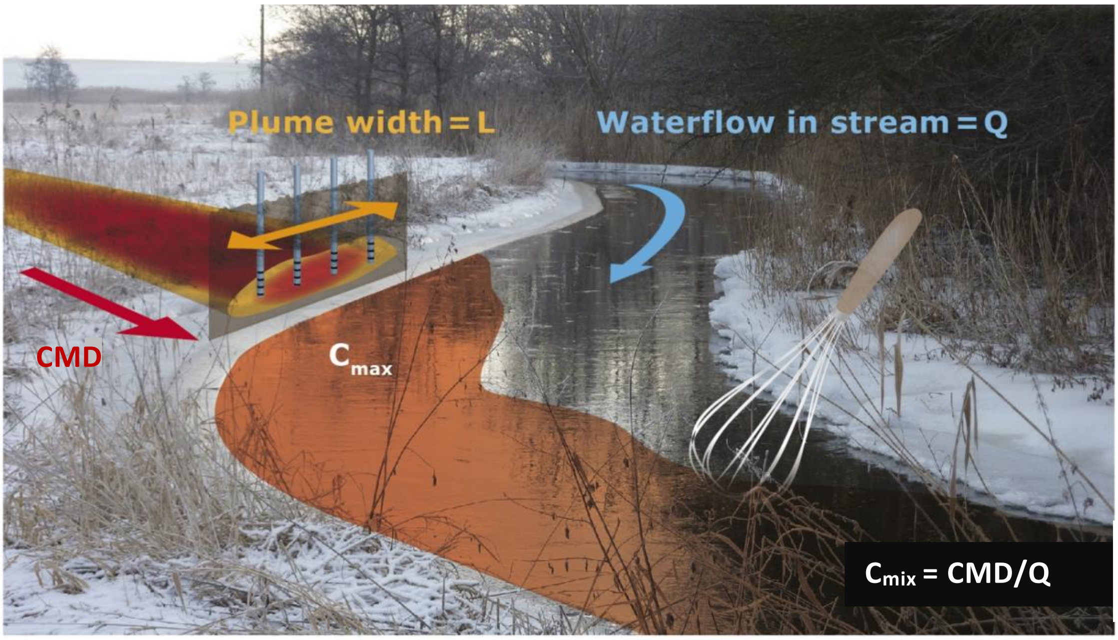

Figure 7. Illustration of the quantification approach of an in-stream estimation of a CMD from a point source simply by knowing the fully mixed SW concentration, C_{mix}, and the stream flow, Q. The example is shown for a contaminant groundwater plume entering a stream. Figure modified from Bjerg et al. (2014).

3.7 Predicted ecotoxicity

The toxic unit approach carried out in this study was applied to obtain an initial overview of potential ecological risk (Tomlin, 2001) of the dissolved-phase chemical concentrations detected in the SW, HZ and back-calculated for the PW. The freshwater crustacean *Daphnia magna* was used as the test organism. The employed sum of TU (ΣTU) for each compound group (i.e. chlorinated ethenes, BTEX, pharmaceuticals, metals) was based on the assumption of additive toxicity and thus neglects potential synergistic and antagonistic effects occurring between the compounds. It was not possible to find the acute effect (immobilization) values (IC50/EC50/LC50) for daphnids for all the detected pharmaceuticals in Grindsted stream. Therefore, an estimate was made for each compound using the Danish (Q)SAR Database. The estimation was based on two different approaches (Leadscope and SciQSAR). The first approach classifies the compounds by structural features using a library of approximately 27,000 structural features (functional groups, heterocycles and pharmacophores) and the physico-chemical descriptors (logP, molecular weight, number of hydrogen bond acceptors and donors) to estimate an LC50 value. The second approach, in contrast, operates with various molecular descriptors (e.g. physical-chemical, electrotopological state, hydrogen electrotopological indices, connectivity indices) to estimate a value. The most conservative estimate was selected and used as a part of risk assessment as suggested by McKnight et al. (2015) (Sonne et al., **II**).

3.8 EQS and threshold values

The EQS values for freshwater metal concentrations that were used in this study were derived from the Common Implementation Strategy for the WFD (2000/60/EC; WFD, 2011). Each EQS value is derived from the lowest short-term (LC50/EC50) value of the three freshwater taxonomic groups: algae, crustaceans and fish from three different trophic levels. The value is then divided by a conservative assessment factor, typically 10,000. The EQS values used for the trace metals were from the Danish EPA (2010). The values of Ba, Ni and Zn were further adjusted with natural background levels. Freshwater sediment benchmarks from the US EPA (2006) were employed to represent BS concentrations for which a chronic exposure resulted in non-lethal endpoints of sensitive invertebrate species.

3.9 Multivariate statistical analysis

Principal Component Analysis (PCA) was employed to obtain an overview of the similarity of the quality of the stream compartments (SW, HZ, BS) along the stream corridor by quantifying the relationships between the detected compounds. Changes in toxic potential and redox sensitive parameters in the HZ were also explored by PCA. All detected concentrations were log-transformed in order to ensure normal distribution. Hierarchical cluster analyses (HCA, SimProf; $p<0.05$) and one-way analysis of variance (ANOVA, Bonferroni t-test or Kruskal-Wallis on ranks; $p<0.05$) were used to capture a significant relationship between the stations.

Non-metric multidimensional scaling (nMDS) was used to detect possible changes in the macro- and meioinvertebrate communities sampled in April and August 2014, respectively. Possible variations in the macroinvertebrate community due to habitat were further explored by comparing the nMDS plots for all habitats (all BS types) with taxa preferring sandy and organic matter habitats. In order to emphasize the dominance of rare taxa, abundance data was fourth-root transformed before similarity matrices were built using Bray-Curtis similarity, and ordination was run 100 times. Additional analyses were performed for the sediment-dwelling and the sensitive Ephemeroptera (mayflies), Plecoptera (stoneflies) and Tricoptera (caddisflies) (EPT) taxa of the macroinvertebrate communities. Both the meioinvertebrate (all taxa) and nematode communities were analyzed. Potential clusters of the stations based on the composition of the invertebrate communities (taxa and abundance) were explored by Hierarchical cluster analyses (HCA, SimProf; $p<0.05$).

All statistical analyses were performed in PRIMER (Clarke and Warwick, 2001).

4 Results

4.1 The chemical impact from multiple sources

An assessment of the three stream compartments, SW, HZ, and BS, showed the chemical quality along Grindsted stream (Figure 3) was impacted by a mixture of chemical stressors from multiple sources that entered the stream by different pathways. Stressors include metals, organic xenobiotics, and macroions. In contrast, the general stream water quality, nutrients, and oxygen demand were all indicative of a healthy stream (Sonne et al., **II**; Rasmussen et al., **I**).

Based on typical national values (90% quantile) and conservative thresholds (EQS, benchmark values), the primary impairment was found in the SW and HZ, while the BS was only slightly affected along the stream corridor. The key contributors were identified as the diffuse metal sources of both geogenic and anthropogenic origin and the groundwater plume from the contaminated site, Grindsted factory. The notably high dissolved aluminum (Al) concentrations further indicated the diffuse metal sources were enhanced by acidification of the noncalcareous deficient aquifer. The main pathway from the key sources to the stream was groundwater discharge.

Overall, the metal stressor decreased the chemical quality of both the SW and HZ. The discharging contaminant plume, however, primarily impacted the chemical quality in the HZ. The impact was multifaceted; high concentrations of xenobiotics and strongly reduced water reduced the HZ water quality. The strongly reduced water was characterized by high concentrations of iron and manganese (metabolic byproducts) generated by microbial degradation of the organic xenobiotics in the plume (Christensen et al., 2000). The groundwater discharge of the high dissolved iron concentrations into the well-aerated stream caused a noticeable precipitation on the BS. The ochre precipitation was especially pronounced in the upstream part of station 4 and at stations S4, 6 and S5 as seen in Figure 8. The iron-reduction process induced an additional, enhanced geogenic release of arsenic from the aquifer, resulting in notable high HZ concentrations (Sonne et al., **II**).

Figure 8: Aerial photo of Grindsted stream in the area of the sampling stations in August 2012 (S4 and S5; Rasmussen et al., **I**), and in April 2014 (St. 4 and 6) and August 2014 (4DS, 4US and 6DS) (Sonne et al., **II** and **III**).

The impairment showed a marked division (PCA analysis) of the sampling stations along the stream corridor based on the detected HZ concentrations of both organic and inorganic contaminants. The chemical data from April and August 2014 showed the inflow zones were narrow, with both spatial and temporal variations in compound composition. Thus, the strongly reduced redox conditions showed a more stable representation in the plume discharge than the contaminants. It was not possible to show a significant division based on the high but evenly distributed trace metal concentrations alone (Sonne et al., **III**). However, the toxic potential (logΣTU) supported that the diffuse metal sources played a substantial role in the stream quality in both the SW and HZ. The concentrations were at high risk levels throughout the investigated stream corridor ranging from -1.78 to 0.37 according to the threshold values given by both Liess et al. (2008, logΣTU≥-3), and von der Ohe and Liess (2004, logΣTU≥-2). In contrast the presence of the groundwater-borne organic xenobiotics (i.e. chlorinated ethenes, BTEX and pharmaceuticals) only showed a significant toxic potential in the HZ at the main discharge zones (Sonne et al., **II**).

The detected organic xenobiotic concentrations in the SW revealed a significant division of the investigated stream stretch depending on the contamination level. Notably, this clustering of the stations remained identical for both April and August 2014 despite the temporal variations present in the composition of the chemical mixture (Sonne et al., **III**).

The combined evaluation of the quantification of the CMD from the diverse anthropogenic point sources (e.g. WWTP, industrial discharge) and the in-stream values (CMD=C*Q) along the stream corridor demonstrated to be a useful tool to displayed both the sources mutual importance and unidentified sources (Sonne et al., **II**). It furthermore showed that the contaminated site discharged several 100 kg/y of both chlorinated ethenes, sulfonamides and barbiturates to the stream. The temporal survey of the chemical SW quality furthermore revealed the total CMD from the groundwater plume (i.e. PCE equivalent) varied very little over time (<10%; Rønde et al., 2017). This indicated that the groundwater discharge was constant.

4.2 Hydromorphological characterization

The characterization of the hydromorphological quality (DHQI) at each sampling station (2012 and 2014) were a similar standard ranging from moderate to good, except at stations 2 and 11 (Rasmussen et al., **I**; Sonne et al., **III)**. Station 2 was characterized by particularly poor in-stream parameters (absence of vegetation and physical variations in the streambed). In contrast station 11 had an overall poor score as this stream section was heavily channelized. For both of these stations, a low diversity of both the meio- and macroinvertebrate community was observed (Sonne et al., **III**; Appendix Table S1). The two stations were, based on these observations, excluded from the invertebrate surveys (Sonne et al., **III**).

4.3 Detected ecological changes

In August 2012, the ecological assessment of the macroinvertebrate community in Grindsted stream showed a reduction of taxonomical density and diversity for the sediment dwelling taxa (nMDS analysis) at the sampling stations located in the primary groundwater plume discharge zones (at stations S4-5; Figure 3). Despite the generally few individuals detected/present in the stream, the stations could be distinguished from the upstream control stations and the stations along a downstream dilution gradient in the SW. However, the macroinvertebrate index, documented to be sensitive to organic xenobiotic contamination in the SW, was not able to identify any critical changes in the community. Morphological (mandible) deformities of the sediment-dwelling Chironomidae larvae were found in the urban area at two stations; one in the area of the main discharge zones (station S5) and one in the upstream part of the town (S6; Figure 3). Due to a low presence in Grindsted stream, it was not possible to conclude significant effects from the contaminated groundwater discharge (Rasmussen et al., **I**).

The eutrophication sensitive indices both showed good ecological quality throughout the stream corridor. The pesticide sensitive index indicated minimal pesticide impact at the time of the campaign (Rasmussen et al., **I**).

The ecological assessment of the benthic invertebrate communities (nMDS analysis) in 2014 showed a significant decrease in abundance in all taxa in the meioinvertebrate community in the area of the primary discharge zones (Figure 9A). A similar decline was seen in the nematode genera although it was not statistically significant. However, the NemaSPEAR indices sensitive to metal and organic xenobiotic contamination in the BS did not indicate an impact (Appendix Table S2). In contrast the macroinvertebrate community (Figure 9B) in April 2014 only showed to be controlled by the habitat of the BS (sand, gravel or organic matter) along the investigated stream corridor (Appendix Figure S2) (Sonne et al., **III**).

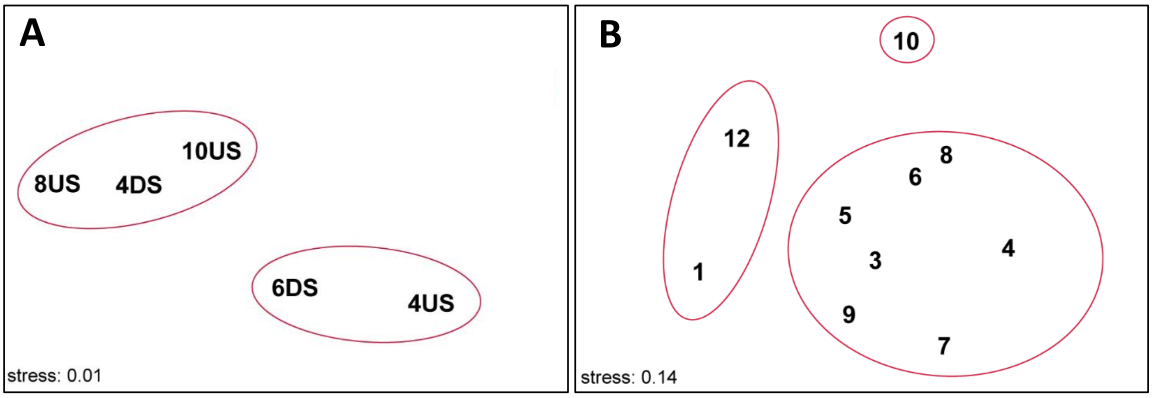

Figure 9: nMDS plots based on the (A) meio- and (B) macroinvertebrate communities (all taxa) sampled in Grindsted stream, 2014. The small meioinvertebrate sampling stations (4DS, 4US, 6DS, 8US, 10US) were conducted in the large macroinvertebrate sampling stations 4, 6, 8 and 10. For locations and details see Figure 5 and section 3.4. Modified figure from Sonne et al. (**III**).

4.4 The overall risk assessment of Grindsted stream

The statistical evaluation of the chemical and biogeochemical quality along the stream corridor in the HZ compartment (PCA and HCA analysis) interestingly revealed significant changes in the meioinvertebrate community (Figure 9A). The decrease of the ecological quality corresponded to a significant reduction in the water quality of the HZ (Sonne et al., **III**). However, at the small meio-invertebrate sampling stations where the compound concentrations were represented by a point measurement, the ecoresponse showed consistency specifically with the strongly reduced conditions in the HZ (Table 2). It did not correspond to the presence of organic xenobiotics, which were only identified signified for one (4US) of the two ecologically impacted stations (4US and 6DS) (Table 2). Chemical stress at this particular station only was further supported by a low $NemaSPEAR_{genus}$[%]-index (Appendix Table S2). However the reduced conditions were also correlated with the presence of increased values of the two plume indicators, lithium and bromide, which indicated that the reduced condition was related to the degradation of the factory plume (Sonne et al., **III**).

Table 2: Significant grouping of the sampling stations in August 2014 are shown according to the water quality in the hyporheic zone and the ecological quality. The separation based on the water quality are shown for the detected concentrations of the (i) organic xenobiotics (i.e. chlorinated ethenes, BTEX and pharmaceutical compounds), the (ii) redox sensitive parameters (dissolved nitrate-N, manganese (Mn), iron (Fe) and sulfate-S), the plume-indicator compounds (bromide, lithium), and dissolved arsenic (As). Note "high" and "low" refer to the contamination level of the xenobiotics - and to the "high" dissolved HZ concentrations of Mn, Fe and As due to strongly reduced conditions. The significant decrease in the ecological quality (highlighted in red) is shown for the abundance of the meioinvertebrate communities (all taxa). For locations of sampling stations see Figure 5.

August 2014			
Station Area: 5m x 5m	Xenobiotics	Redox	Meio Total abundance
-	-	-	-
10 US	Low	Low	1114
-	-	-	-
8 US	Low	Low	3765
-	-	-	-
6 DS	Low	High	312
-	-	-	-
4 US	High	High	298
4 DS	Low	Low	1931
-	-	-	-
-	-	-	-

At the large macroinvertebrate sampling stations in 2012 (stations S4 and S5), however, it was not possible to distinguish between the significant presence of organic xenobiotics and strongly reduced conditions in the HZ when revising the areas based on the current data from Grindsted stream (Figure 8; Figure S7 in Sonne et al., **III**).

The significant impairment to the ecological quality of the two invertebrate communities, however, was not possible to detect using the traditional assessment of the chemical SW quality regardless the approach (PCA, TU, ecological indices). The good ecological quality throughout the stream stated by the sensitive SW eutrophication indices was in good correlation with the assessment of the general stream water quality, nutrients, and oxygen demand in Grindsted stream.

5 Discussion

The present PhD study showed that the assessment of a mixed land use stream system could be improved by application of a methodology, where the different stream compartments, the two communities of the macro- and meioinvertebrates and linkage to sources are strengthen.

The CMD approach was a useful tool that provided an initial ranking of the known sources and uncovered contaminant sources in the stream system. Furthermore, the approach established that the contaminated plume discharges hundreds of kilograms of chlorinated ethenes and sulphonamides into the stream every year. Thereby it demonstrated the importance of including large contaminated sites in the risk assessment of mixed land use stream systems and valuable information can be obtained from the in-stream CMD approach (Sonne et al., **II**).

The investigation further showed a significant impairment of the invertebrate communities documented in the primary discharge zones of the contaminant plume. Based on the distinct link between the strongly reduced conditions in the HZ and the response of the meioinvertebrates we suggest that this is a result of a cause-effect relationship between the chemical stressors, organic and inorganic, in the system. An interaction mechanism that occurs outside the invertebrates: as the degradation of organic xenobiotic compounds in the plume causes reduced conditions in the aquifer which induces geogenic release of reduced iron (and e.g. arsenic). This chain-reaction thus leads to increased HZ concentrations and bioavailability of toxic elements in additional to the organic xenobiotics in the primary discharge zones of the contaminant plume. It is therefore not possible to determine this by a dose-response relationship of the organic xenobiotic mixture effects. Neither is it possible to predict the observed ecological response despite application of additional safety factors to the mean LC50 (EC50/IC50) values of *D. magna*, i.e. accounting for acute (LC50/10) and chronic (LC50/1000) effects on the invertebrates, as suggested by Malaj et al. (2014) (Table 3). Instead, the predicted toxic potential in the HZ of the six meioinvertebrate sampling stations continued the pattern of giving a confounding result that was not in accordance with the actual observed changes within the community.

Table 3: Adapted predicted toxicity values ($TU=C_i/LC50$) estimated for the organic xenobiotics detected in the hyporheic zone, using LC50 values for the crustacean *D. magna*, which correspond to the suggested conservative approach: acute risk threshold (ART: $1/10^{th}$ of C_i, indicated as LC50/10) and chronic risk threshold (CRT: $1/1000^{th}$ of C_i, LC50/1000). Also shown are the $\log\Sigma TU$ values for the original TU calculation (LC50).

Sampling station (August 2014)		2DS	4DS	4US	6DS	8US	10US
Pharmaceuticals (HZ)							
LC50	Log(ΣTU)	-7.00	-3.61	**-1.48**	-5.61	-4.14	-4.38
LC50/10	Log(ΣTU)	-7.00	**-2.61**	**-0.48**	-4.61	-3.14	-3.38
LC50/1000	Log(ΣTU)	-7.00	**-0.61**	**1.52**	**-2.61**	**-1.14**	**-1.38**
CAHs (HZ)							
LC50	Log(ΣTU)	-6.12	-3.51	**-1.24**	-3.60	-4.42	-5.46
LC50/10	Log(ΣTU)	-5.12	**-2.51**	**-0.24**	**-2.60**	-3.42	-4.46
LC50/1000	Log(ΣTU)	-3.12	**-0.51**	**1.76**	**-0.60**	**-1.42**	**-2.46**
BTEX (HZ)							
LC50	Log(ΣTU)	-3.88	-3.89	**-2.53**	-3.98	-4.14	-4.11
LC50/10	Log(ΣTU)	**-2.88**	**-2.89**	**-1.53**	**-2.98**	-3.14	-3.11
LC50/1000	Log(ΣTU)	**-0.95**	**-0.89**	**0.47**	**-0.98**	**-1.14**	**-1.11**

The significant ecoresponse further indicates that a prolonged impact from a strongly reduced environment characterized by high iron and manganese concentrations could be damaging to the organisms that reside in the BS. Thus, this impairment was observed at stations where especially the HZ dissolved iron concentrations were particularly high (14-21 mg/l) compared to the remaining stations (0.1-6 mg/L). As the stream was well aerated (oxygen-rich), the alteration in redox conditions will endorse the presence of both solid and dissolved iron species in the interface between the HZ and the upper BS (Linton et al., 2007). However, regardless of the complexity, studies of its toxic properties in freshwater systems have indicated that both precipitated and dissolved forms can have direct and indirect effects on the invertebrate communities (Hjorth et al., 2016; Höss et al., 2015a-b; Linton et al., 2007; Vuori, 1995; Wellnitz et al., 1994). Consequently, the iron species could be important and possibly play a central role in the significant impact found in the invertebrate communities within the primary discharge zones of Grindsted stream.

The continuous source of groundwater–borne contaminants into the stream resulted in high measureable SW concentrations that were unique for this stream site (Conant et al., 2004; Weatherill et al., 2014). The SW contamination was persistent in the water column at concentrations (4-1.2 µg VC/L) for more than 7 km downstream of the main discharge zones. Yet, no direct link between the VC concentration and the ecological quality of the invertebrates was found in this study, although sub-lethal long-term effects have been documented at concentrations as low as 0.1 µg VC/L to the macroinvertebrate *D. magna* by Houde et al. (2015). This could be explained by a weak impact on the macroinvertebrates combined with re-colonization from the healthier, upstream corridor section (Stoll et al., 2016).

This study further illustrates that solitary assessment of the SW compartment, as promoted by the WFD, would fail to detect/identify the ecological impact at this stream site. By constraining the chemical assessment to one or two compartments, we suspect that the actual risk for such stream systems has been underestimated. Thus, we recommend a broad focus that includes heavy metals, organic compounds, and inorganic compounds in multiple compartments in future monitoring programs of mixed land use stream systems. Furthermore it demonstrated that the meiofauna community, a rarely used ecological tool, can add valuable information to the link between the chemical and the ecological stream quality in not just the BS (Höss et al., 2011) but also the HZ.

6 Conclusion

The focus of this thesis was to investigate and develop the interpretation of the ecological quality and the presence of multiple chemical stressors in mixed land use stream systems.

- The literature overview for field-based risk assessment approaches currently applied in mixed land use stream systems indicates that attention is being given to the many of the various sources found in such systems, with a preference for investigating the surface water and suspended and bed sediment compartments. These investigations, however, do not cover contaminated sites where chemical impacts on streams are known to occur via the groundwater pathway. As such, the reduction in hyporheic water quality represents a potential missing link in both the chemical and ecological risk assessments for these systems.

- Field studies were performed at the Grindsted stream in the western part of Denmark. It is a groundwater-gaining stream in a catchment representing a mixed land use system with urban and agricultural activities and hosting two large contaminated sites. The results showed:
  - The contaminant mass discharge approach was an effective tool to quantify the chemical stressors and ascertain the known anthropogenic point sources' mutual importance, unidentified sources (e.g. road salting) and stressors (e.g. pharmaceuticals from fish farm practice), solely based on water concentrations and flow. The contaminant mass discharge estimations thus provide valuable information for the management of streams in mixed land use catchments and future remedial actions to improve the stream quality.
  - The combined evaluation of the chemical quality in the stream water, hyporheic zone and bed sediment using the contaminant mass discharge framework, threshold values, and toxic potential made it possible to link the detected chemical compounds to their source/origin and determine the toxic potential for most compounds to the stream ecosystem.
    - It showed that the groundwater-stream water interaction was the key/main pathway for chemical stressors to enter the groundwater-fed stream.

- It documented the significance of the diffuse geogenic and anthropogenic metal sources and the associated risk throughout the investigated stretch to the stream ecosystem in both the stream water and hyporheic zone.
- It demonstrated the importance of the contaminated site with a large plume as a significant source of chemical hazard to a stream system.

- The contaminated groundwater plume discharge impacted both the organic and inorganic chemical quality in the hyporheic zone due to the degradation of the organic xenobiotics e.g. BTEX,N in the plume. This enhanced geogenic mobilization of especially iron and arsenic. The impairment had significant local importance for the chemical quality in the hyporheic zone at the main discharge zones of the plume.
- The simultaneously chemical assessment of the three stream compartments further gave valuable insight to key patterns along the stream corridor regardless of whether or not the toxicity of an assessed compound was known. Thus, it provided a holistic understanding and potential links to observed changes in the ecological quality.
- It was not possible to link the evenly distributed, critically high, toxic potentials of the metals throughout the investigated stream stretch to an ecological change in the invertebrate communities. However, local changes in the biogeochemistry due to the degradation of the contaminant plume (e.g. high water concentrations of iron and arsenic in the hyporheic zone) were demonstrated to generate a significant stress to stream invertebrates in the main discharge zones.
- The observed response in the invertebrate communities strongly implies that the discharge of the contaminant plume causes small windows of high impact zones in the hyporheic zone and the connected bed sediment. Continuous inflow from the plume had especially negative impact on the organisms embedded in the streambed of these windows. Because it was a combined effect caused by both organic and inorganic chemical stressors it was not possible to predict the impact stressor by stressor or compound group by compound group as previous chemical risk assessments have suggested in published literature.
- The meioinvertebrate community demonstrated to be sensitive to the chemical water quality of the hyporheic zone while the macroinvertebrates showed less sensitivity. This study emphasizes the importance of

including both the meio- and macroinvertebrate communities in an ecological assessment, as it enables us to link ecological quality to the chemical quality in all three stream compartments: stream water, hyporheic zone and bed sediment.

Finally we believe this holistic approach, where chemical assessment is conducted in the individual compartments and combined use of invertebrate communities, is essential in uncovering key patterns and impact drivers in a mixed land use stream system.

7 Future perspectives

The PhD project dealt with the risk assessment of the chemical and ecological quality in a mixed land use stream system. The following perspectives and issues for future research and investigations have been identified:

- For future investigations it is relevant to apply the developed approach at mixed land use stream systems in other areas with different sources, and hydro(geo)logical conditions. It would also be beneficial to include emerging compounds in the analytical program in future studies. An example is the bio-accumulative and extreme stable perfluorinated chemicals (ASTSWMO, 2015), which have very low freshwater EQS such as the perfluorooctane sulphonates (PFOS; WFD EQS, 2011).

- The discovery of ecological impact from the long-term discharge of a strongly reduced plume indicates that all contaminant plumes, which are reduced because they undergo degradation, are a risk to groundwater-fed stream ecosystems. This will be despite of the toxic potential of the xenobiotic compounds in the plume. Thus also organic and inorganic plumes with NVOC and ammonium strongly represented in landfills can pose an additional risk to stream ecosystems. These findings would bring old landfills close to streams without liners or leachate collection under renewed attention.

- The demonstrated sensitivity of meioinvertebrate communities to the chemical stream quality furthermore enables the opportunity of a broad standardization of the characterization of the ecological health across borders, e.g. in the European Union, as the meioinvertebrates are ubiquitously distribution throughout all ecoregions (even in streams dominated by soft BS) (Kennedy and Jacoby, 1997; Wolfram et al., 2012). This is a tool that could moreover help reduction of unclear ecological results – inconsistencies inherent to e.g. the macroinvertebrates community structure which is only comparable across the northern part of the hemisphere (Statzner & Bêche, 2010).

- The investigation and possible confirmation of the meioinvertebrate community' sensitivity to SW contamination due to their important role in both benthic and epilithic habitats (Majdi et al., 2017; Peters et al.,

2007) could thus strengthen their ability to support a standardization of ecological assessments across ecoregions.

- Explore new possibilities/approaches such as ecotoxicogenomics tools to risk assess the ecological health due to the chemical quality in the SW. Instead of measuring whole-organism responses (LC50/EC50 values) these techniques investigate/explore sub-lethal effects on a cellular level. The early warnings are identified by isolating the active RNA from live organisms cultured and exposed to the undiluted SW. This laboratorial approach seems promising as it can accounts for the co-exist of multiple chemical stressors and the possibility to interact in the SW. Some of the genotoxity indicators have even shown to be contaminant point source specific (Oberholster et al., 2016). This thus has potential to assist identification of key sources and the risk assessment of a mixed land use stream system on a catchment scale.

- For practical use of our holistic risk assessment approach we suggest to pre-screen the properties of the stream system the hydrological settings and size, and the geology and land use in the catchment area. In this way it will provide an overview of the probable key contaminant pathways to the stream and potential impacts of the chemical sources relevant/important for the risk assessment. This would thus support the understanding of which stream compartment that are potentially at risk and help focus the investigation strategy to relevant compartments and communities in order to optimize the field campaign resources.

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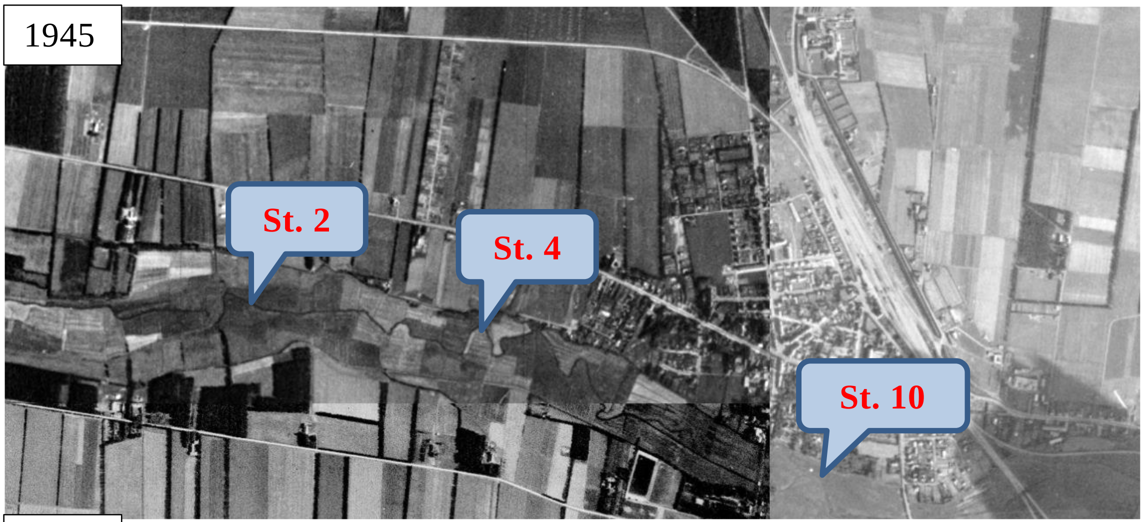
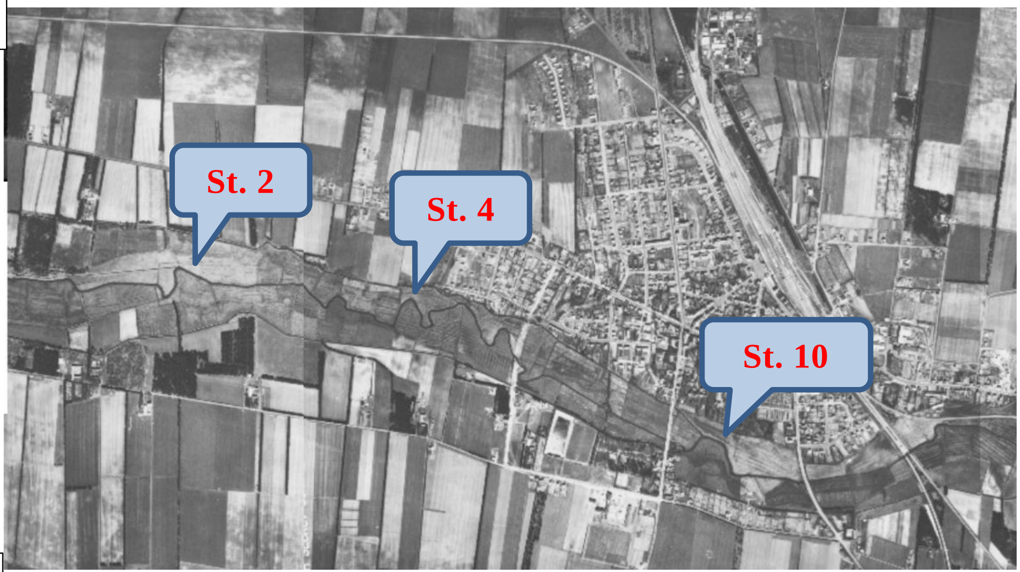
9 Appendix – Supplementary information

Figure S1: Arial photos of the study site in Grindsted stream in 1945, 1954, 1976, 1985, 1990, and 2013. The locations of the stations 2, 4 and 10 in 2014 are highlighted.

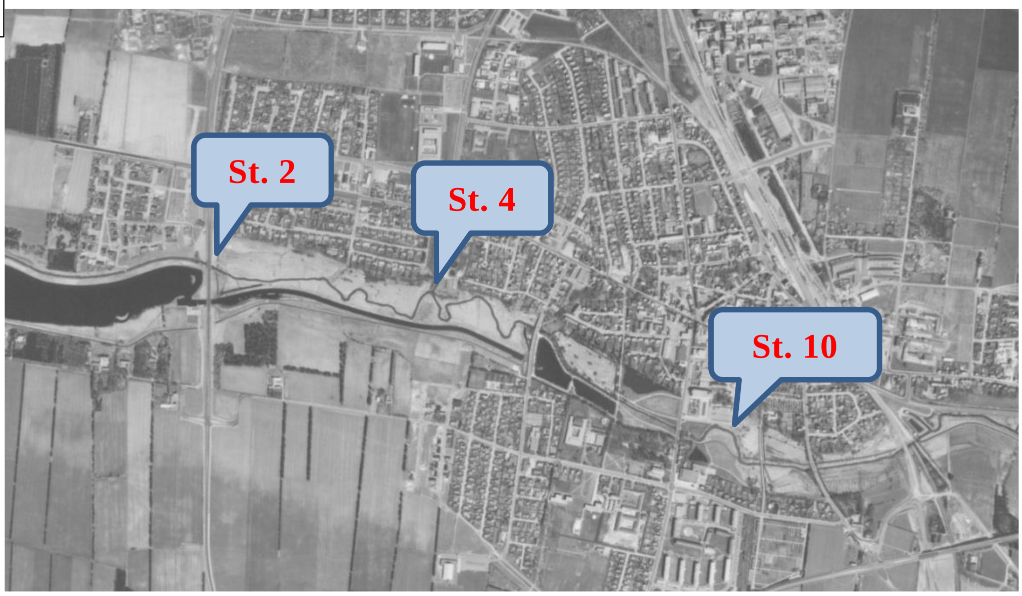
1945



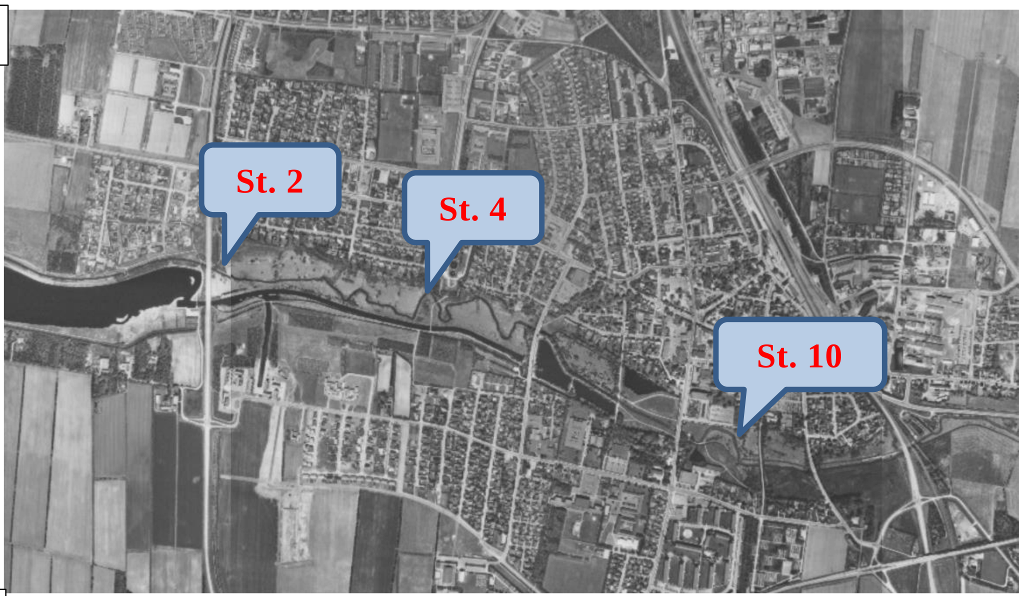
1954



1976



1985



1990

2013

Table S1: Taxon and abundance for the twelve macroinvertebrate sampling stations in April 2014.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Tubificidae indet	0	0	0	0	0	0	0	0	3	1	0	0
Physa fontinalis	0	0	0	0	0	0	0	0	0	0	0	0
Lymnaea peregra	2	0	0	0	0	0	1	0	0	0	0	4
Ancylus fluviatilis	0	0	0	0	0	0	0	0	0	0	0	0
Potamopyrgus antipodarum	0	0	0	0	0	0	0	0	2	0	0	0
Pisidium sp.	1	4	4	1	0	3	3	9	9	20	0	0
Oligochaeta indet	108	166	166	73	102	141	79	148	115	34	24	125
Glossiphonia complanata	0	1	1	0	1	1	0	1	0	2	0	0
Glossiphonia concolor	0	2	2	2	0	7	0	0	3	0	3	0
Erpobdella octoculata	0	0	0	0	1	1	0	3	1	2	1	1
Hydracarina indet	14	1	1	6	7	2	0	8	3	11	4	14
Ostracoda indet	0	1	1	0	0	0	0	0	0	0	0	0
Gammarus pulex	544	254	254	237	140	191	268	157	148	427	133	320
Asellus aquaticus	9	4	4	2	3	1	0	2	11	7	9	2
Procloeon sp.	0	0	0	0	0	0	0	0	0	1	0	0
Leptophlebia sp.	0	0	0	0	0	0	0	0	0	1	0	0
Leptophlebia marginata	1	0	0	0	0	0	0	0	0	1	0	0
Paraleptophlebia submarginata	1	0	0	0	0	0	0	0	0	0	0	0
Baetis sp.	4	7	7	0	12	5	1	3	1	24	3	7
Baetis rhodani	14	37	37	14	67	35	12	46	13	33	29	30
Caenis rivulorum	1	0	0	0	0	0	0	0	0	0	0	0
Amphinemura sp.	0	0	0	0	0	0	0	0	0	0	0	5
Isoperla grammatical	0	0	0	0	0	0	0	0	0	0	0	0
Isoperla difformis	4	0	0	0	0	0	0	0	0	0	2	0
Perlodidae indet	2	0	0	0	0	0	0	0	0	0	0	0
Protonemura mayeri	0	0	0	1	0	0	0	0	0	0	0	0
Protonemura sp.	0	0	0	0	0	1	0	0	0	0	0	0
Isoptena sp.	0	0	0	0	0	0	0	0	0	0	0	0
Nemoura avicularis	0	0	0	0	0	0	0	1	0	0	0	0
Nemoura sp.	0	0	0	0	0	0	0	0	0	1	0	12
Leuctra sp.	0	0	0	2	4	1	0	0	1	7	0	0
Corixinae sp.	0	0	0	1	0	0	0	0	0	0	0	0
Elmis aenea	0	0	0	0	0	0	0	0	0	1	0	4
Oreodytes sanmarkii	1	2	2	0	2	1	0	0	1	0	0	2
Oreodytes seidlitz	0	0	0	0	0	3	9	1	0	0	2	11
Oreodytes sp.	0	0	0	0	0	0	0	0	0	0	0	1
Platambus maculatus	0	0	0	0	0	0	0	0	0	0	0	0
Dytiscidae indet	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophiloidea indet	0	0	0	0	1	0	0	0	0	0	0	0

Helodes sp.	0	0	0	0	0	0	0	0	0	0	0	1
Sialis nigripes	0	0	0	0	0	0	0	0	0	0	0	0
Sialis lutaria	0	0	0	0	0	0	0	0	0	4	0	0
Plectrocnemia conspersa	0	0	0	2	0	0	0	0	1	5	0	0
Phryganeidae indet	0	0	0	0	0	0	0	0	0	0	0	0
Rhyacophila sp.	0	0	0	0	0	0	0	0	0	1	0	4
Rhyacophila nubile	0	0	0	0	0	4	0	3	3	4	0	7
Hydroptilidae indet	5	4	4	1	4	3	1	11	7	3	2	0
Siltalai sp.	0	0	0	0	0	0	0	0	0	0	0	1
Hydropsyche pellucidula	1	1	1	0	1	1	0	0	0	1	0	0
Polycentropodidae indet	0	0	0	0	0	0	1	0	0	0	0	0
Polycentropus sp.	0	0	0	0	0	1	0	0	0	0	0	0
Polycentropus irroratus	1	0	0	0	0	0	0	0	0	0	0	0
Polycentropus flavomaculatus	0	0	0	0	0	1	0	0	0	0	0	0
Holocentropus plectrocnemia conspersa	0	0	0	0	0	0	2	0	0	7	0	0
Halesus sp.	1	0	0	0	0	0	0	0	0	1	0	1
Halesus radiates	1	1	1	0	1	0	0	0	7	0	1	0
Politus sp.	0	1	1	1	0	1	4	5	0	1	0	0
Chaetopteryx villosa	0	0	0	0	0	0	0	0	5	0	0	0
Limnephilidae indet	0	0	0	0	0	0	1	0	0	0	0	12
Limnephilus rhombicus	9	3	3	13	4	4	13	9	5	86	2	0
Ecclisopteryx dalecarlica	0	0	0	1	0	1	1	3	6	3	0	62
Potamophylax sp.	0	0	0	0	0	0	0	1	1	0	0	0
Potamophylax latipennis	0	2	2	1	1	3	0	0	0	0	0	2
Brachycentrus sp.	1	0	0	0	0	0	1	0	2	0	0	0
Brachycentrus maculatum	2	0	0	0	0	1	0	0	0	0	0	0
Atherix sp.	0	2	2	0	0	0	0	0	2	0	0	0
Simuliidae indet	1384	54	54	1	78	248	34	40	25	89	344	296
Chironomidae indet	4	0	0	0	0	0	0	1	0	3	1	0
Chironominae chironomini	0	0	0	0	0	58	0	11	0	38	0	7
Chironominae tanytarsini	10	92	92	41	21	17	22	33	39	96	10	2
Chironominae orthocladiinae	5	11	11	23	65	53	56	34	2	118	10	48
Chironominae tanypodinae	16	11	11	15	26	14	19	19	24	117	30	1
Prodiamesinae olivacea	14	4	4	0	13	0	14	0	6	1	7	0
Chironominae chironomus sp	0	0	0	0	0	0	0	0	0	12	0	0
Hemerodromia sp.	0	0	0	0	0	0	0	0	0	0	0	0
Empidiae indet	0	0	0	1	2	2	0	0	4	1	0	0
Tipuloidea indet	0	0	0	3	0	13	4	1	0	15	8	3
Pediciinae Dicranota sp.	5	8	8	9	2	11	17	15	3	12	4	7
Ceratopogonidae indet	0	10	10	0	0	0	8	0	1	1	6	0

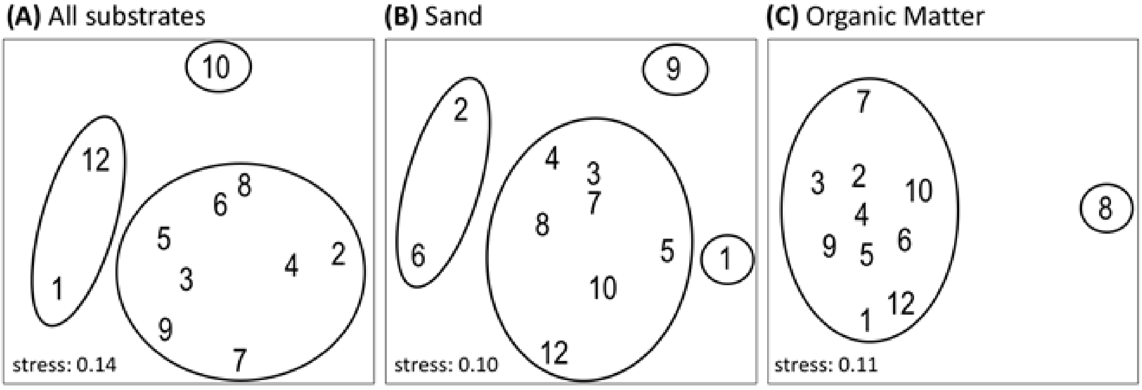


Figure S2: nMDS plots for all taxa of macroinvertebrate community living at streambed sediment predominated by A) all sediment types (sand, organic matter, gravel), B) sand and C) organic matter recorded in April 2014. Red circles indicate clusters that differ significantly from each other (HCA SimProf $p<0.05$).

Table S2: List of nematode indices results for NemaSPEAR$_{metal}$ and NemaSPEAR$_{organic}$. The lower the number the more stress. The threshold is ~30.

Station	NemaSPEAR	
	Metal	**Organic**
4DS	35.5	43.1
4US	41.8	48.3
6DS	24.8	38.3
8US	18.4	40.8
10US	9.7	30.6

10 Papers

I. Rasmussen, J.J., McKnight, U.S., Sonne, A.Th., Wiberg-Larsen, P., Bjerg, P.L., 2016. Legacy of a Chemical Factory Site: Contaminated Groundwater Impacts Stream Macroinvertebrates. Arch. Environ. Contam. Toxicol. 70, 219-230.

II. Sonne, A.Th., McKnight, U.S., Rønde, V., Bjerg, P.L., 2017. Assessing the spatial chemical contamination dynamics of a mixed land-use stream system. Submitted.

III. Sonne, A.Th., Rasmussen, J., Höss, S., Traunspurger, W., Bjerg, P.L., McKnight, U.S., 2017. Re-thinking stressor interaction: novel insights advancing stream ecosystem impact assessments. Manuscript in preparation.

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