Removal of Pharmaceuticals at Strömsund Sewage Treatment Plant by the E-peroxone process

Results from a pilot-study financed by Naturvårdsverket

Final report

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SAMMANFATTNING PÅ SVENSKA

Naturvårdsverket har finansierat denna förstudie för demonstration av en ny avancerad oxidationsprocess för rening av läkemedelsrester i avloppsvatten. Relevansen av att studera olika reningstekniker för läkemedelsrester har ökat på senare år genom nya kunskaper om hur läkemedel sprids, uppträder och deras potential att ge en rad oönskade effekter i miljön. Kända exempel på störningar som påvisats är bl.a. hormon- och reproduktionsstörning hos fisk och bioackumulation av vissa substanser i den akvatiska näringskedjan där höga halter uppmätts i vävnad hos flera olika organismer. Vidare kan utsläppen av antibiotika potentiellt stimulera utveckling av antibiotikaresistenta bakteriestammar. Det kommer troligen i en snar framtid att införas lagkrav på dylik rening med anledning av det potentiella hot som läkemedelsutsläpp utgör både för miljö- och människors hälsa. Drivkrafter för införande av förbättrad reningsteknik avseende läkemedelsrester är således främst att förbättra skyddet den akvatiska miljön , men även för att skydda vatten som resurs t.ex. för dricksvattenproduktion och förhindra potentiellt skadlig exponering för människor.

Strömsunds kommun i Jämtlands län har varit projektägare med Envix Nord AB (Envix) som teknikleverantör. Envix har utfört projektering, byggnation av reningssystem samt stått för utförande, provtagningar, sammanställning och redovisning av resultaten i föreliggande rapport. Strömsunds kommun har stått för teknikkunnande avseende konventionella reningsprocesser, personella resurser på plats och kunskap om det det aktuella avloppsvatten och därmed utgjort ett värdefullt teknikstöd både under anläggningsfas och utförande. Projektet har pågått under perioden augusti 2021 till februari 2023. Samtliga tester har utförts vid Strömsunds avloppsreningsverk med Ströms Vattudal som recipient. Ströms Vattudal utgör en del av Faxälven, biflöde till Ångermanälven. Strömsunds avloppsreningsverk, centralt beläget i Strömsunds samhälle,är det största reningsverket i kommunen och har nyligen (2017) byggts om och inkluderar även ett biologiskt reningssteg (MBBR).

Det övergripande syftet i projektet var att Strömsunds kommun tillsammans med Envix skulle implementera en ny avancerad oxidationsprocess, elektro-peroxon (E-peroxone) som tilläggsrening till konventionella reningssteg vid avloppsreningsverket och i industriskala (215 m^3/24h) rena avloppsvatten från att oönskade läkemedelsrester. Reningsförsöken har utförts under fyra säsonger (vår, sommar, höst och vinter) för att undersöka reningsgraden under varierande omgivningsförhållanden, flöden och kvalitét på inkommande avloppsvatten. Försöken har utförts med kontinuerlig drift i ett helautomatiserat system med möjlighet till både fjärrövervakning och fjärrstyrning. Som jämförelse har tilläggsreningens effekt jämförts mot behandling med ozon, en annan mer vanlig avancerad oxidationsprocess för läkemedelsrening. Utvärdering har baserats på analyser för ett relevant urval av 98 olika läkemedelssubstanser och 2 benzotriazoler med analys på både obehandlat och behandlat avloppsvatten för beräkning av reningseffekt. Vidare har behandlingseffekten från den avancerade oxidationsprocessen utvärderats med biologiska analysmetoder för att ge integrerade svar på eventuella oönskade effekter från exponering för obehandlat och behandlat avloppsvatten. Analys av både akut toxicitet och subletala effekter har undersökts på genregleringsnivå där grad av störning har studerats för ett urval av relevanta biologiska funktioner rörande reproduktion, hormonell och immunologisk störning samt olika typer av kända stressresponser som aktiveras vid exponering för olika substanser och främmande ämnen. Biologiska analyser har utförts både in vivo och in vitro.

Av 100 analyserade substanser detekterades 43 st vid något tillfälle i orenat avloppsvatten efter de konventionella reningsprocesserna. Baserat på medelvärden uppskattas ca 0,11 kg läkemedelsrester avrinna till recipienten varje dag vilket motsvarar ca 40 kg per år. Skulle alla typer av läkemedel som försäljs i Sverige (ca 1200 substanser) analyseras på motsvarande sätt skulle mängden utsläppta läkemedel öka betydligt, men går i dagsläget inte att kvantifiera. Förstudien har ej haft som syfte att bedöma risker för utsläpp av läkemedelsrester till aktuell

recipient utan kräver separata riktade undersökningar både vad gäller kemisk analys och studier av biologiska effekter i recipientens miljö.

Resultaten från förstudien visar att E-peroxon uppnår en reningsgrad för detekterade läkemedelsubstanser i spannet 86-94 % för alla säsonger. Små säsongsvisa variationer påvisades och metoden fungerade robust under alla förhållanden med mycket hög reningsgrad. Ingen bromatbildning kunde noteras varken vid E-peroxone eller ozonbehandling.

Den akuta toxiciteten sjönk väsentligt genom e-peroxonbehandling jämfört med enbart konventionellt behandlat avloppsvatten. Ca 55 % mortalitet uppmättes i obehandlat vatten vilket reducerade till ca 25 % genom E-peroxonbehandling. Akut toxicitet minskade även som förväntat genom ozonbehandling, men reduktionen var inte lika stor. Vid analys av upp- och nedreglering av specifika gener som utgjort biomarkörer för att påvisa subletala effekter var det svårt att utläsa signifikanta skillnader mellan obehandlat avloppsvatten och behandlade prover.

Sammantaget uppvisar förstudien mycket hög reningsgrad för den implementerade tekniken. Energiåtgången beräknas inte heller skilja sig väsentligt jämfört med ozonbehandling utan det har i förstudien uppvisats uppenbara fördelar med E-peroxone jämfört med ozonering. Den nya tekniken bedöms helt skalbar och nästa steg är att implementera rening i full skala vid lämpligt avloppsreningsverk med behov av läkemedelsrening.

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1 BACKGROUND

A large variety of chemicals of anthropogenic origin can unfortunately be found in the global aquatic environment including Swedish surface waters. One obvious and common point source of these chemicals, including various contaminants, are effluents from municipal sewage treatment plants (STPs) due to its function as a funneling point of contaminants that are spread through the water media of different origin (sewage, wastewater, storm water). Further, conventional treatments in municipal sewage treatment plants have in general limited effectiveness for removing many contaminants including pharmaceutical residues and personal care products. Contaminants that are detected in the environment within the ng/L to µg/L range (or even lower) are often referred to as micropollutants (IKSR-CIPR-ICBR., 2010).

According to treatment of wastewaters and drinking waters, micropollutants can be classified into two sub-classes. These are 1) legacy contaminants (for which toxic effects have been addressed and safety measurements have been taken), and 2) emerging contaminants (most of which are not yet regulated and are thought to pose potential threats to human health and ecosystems) (Klamerth et al., 2009). Current project mainly focused on removal of emerging micropollutants in wastewaters.

Micropollutants have been detected worldwide in surface waters and wastewaters (Barbosa et al., 2016; Gracia-Lor et al., 2012; Lindberg et al., 2014; Loos et al., 2013; Luo et al., 2014; Östman et al., 2017; Ternes, 1998). For example, Loos et al (2013) detected 125 of 156 screened polar organic contaminants in effluents of 90 STPs scattered across the European Union (EU). These results clearly highlight a need of an efficient treatment process for chemically diverse emerging micropollutants. Similarly, Lindberg et al (2014) detected 51 out of 105 pharmaceuticals in the aqueous phase of effluents from a STP in Umeå municipality, Sweden. In another recent mega survey by Wilkinson et al (2022), sampling was conducted in 258 rivers around all continents in 104 countries and pharmaceutical residues were found at all locations at different concentration levels when screened for 61 different types of pharmaceuticals. According to Wilkinson study, at >25% of the sampling sites, detected concentrations for at least one pharmaceutical was greater than concentrations considered safe for aquatic organisms. Highest concentrations of pharmaceuticals were found in low- to middle-income countries and in areas with poorly developed wastewater and waste management infrastructure and areas with pharmaceutical manufacturing units.

The removal of various types of micropollutants including pharmaceuticals in wastewaters has gained increased interest during the last 25 years due to identification of the potential threats that micropollutants may cause to both the environment and human health. Thus, emissions of micropollutants resulting in increased concentrations in aquatic systems may have adverse effects, mainly on aquatic organisms, but have lately been shown to also have implications to the non-aquatic food-web due to biomagnifying properties of some pharmaceuticals (Richmond et al., 2018). For example, in fish, it has been observed cellular changes in several rainbow trout organs caused by carbamazepine, diclofenac and metoprolol (Triebskorn et al., 2007). Another potentially severe issue is the progression of antibiotic resistance related to the occurrence of antibiotics in the environment (Guardabassi et al., 1998; Sengupta et al., 2013). Richmond et al (2018) found that pharmaceutical residues could affect both invertebrates and insects including predators as spiders and eventually can cause magnifying levels in an aquatic top predator as brown trout. Thus, affecting all these species could also contribute to an indirect exposure route for humans who consume fish that contains the pharmaceutical residues.

Another example of pharmaceuticals that accumulate through the food chain are presented in Lagesson et al (2016) who used a small mesocosm for studying five pharmaceuticals for longer periods of time. It was found examples of both diminishing levels of pharmaceuticals in biological tissues followed by water phase concentrations. However, a bioaccumulating trend

was for example found in perch for a commonly found pharmaceutical, i.e., carbamazepine throughout the testing period.

A recent study by Previsic et al (2021) showed that trophic transfer of selected pharmaceuticals and endocrine disruptors can occur from the aquatic food web to the terrestrial food web. This was shown in experiments conducted for caddisflies, an important food source for aquatic predators and that was found to bioaccumulate both pharmaceuticals and endocrine disrupting agents, presumably causing transfer to higher trophic levels both in the aquatic and terrestrial food web. However, the relationship of pharmaceuticals bioaccumulation was indicated to be largely impacted by the insect metamorphosis and feeding behavior, thus making this phenomenon immensely complicated to study for large ecosystems.

There are many well founded arguments for both the scientific community and management authorities for taking appropriate steps towards decisions on preventative measures aiming to reduce amount of pharmaceuticals in water resources. New knowledge is continuously building up, but due to uncertainties and the complex nature of pharmaceuticals and other emerging contaminants in the environment, precautionary strategies are preferable regarding actions in terms of pharmaceutical targeted treatment of sewage wastewaters.

1.1 Sewage treatment plants in Sweden

The establishment of municipal STPs started slowly in Sweden in 1940. By 1955, 30 municipal STPs were operating in the whole country. A major turning point occurred around 1960, when eutrophication and contamination by heavy metals in Swedish waters gained public attention. As a result, the Swedish environment protection agency (SEPA) was established in 1967. The numbers and capacities of Swedish STPs subsequently expanded significantly during the 1970s, resulting in the (continuing) connection of all households in urban areas to STPs. These STPs mainly applied secondary biological treatment for removal of organic matter, and tertiary chemical treatment (primarily for removal of phosphorous). Further development resulted in introduction of another treatment step in the 1990's for removal of nitrogen (Swedish Environmental Protection Agency, 2014). In general, the current STPs in Sweden are not yet equipped with advanced processes to remove micropollutants except for very few, thus acting as hot spots in spread of micropollutants in the environment.

1.2 Non-Conventional treatment techniques for micropollutants removal- Advanced oxidation processes

Advanced oxidation processes (AOPs) and sorption by activated carbon are two processes that have been tested in Swedish STPs as advanced tertiary treatment options for removing micropollutants. Activated carbon has been tested on pilot scale (Kårelid et al., 2017) while AOPs have been developed and tested on medium to full scale due to their effectiveness for oxidizing micropollutants. Recently, several projects funded by the SEPA have finalized their pilot tests using various techniques at other locations from several projects. The outcome has been compiled in a recent report (Havs- och vattenmyndigheten., 2018).

In Sweden, the first small to medium sized advanced oxidation (ozonation) treatment plant, with 12000 population equivalents (PE) capacity, was introduced for removal of pharmaceuticals in 2015 at Knivsta STP (Björlenius, 2018). The first full-scale ozonation facility was built in 2016 at Nykvarn STP, Linköping. This plant has a connected population of 145 200 people, and organic matter load corresponding to 235 000 PE (Tekniska verken, 2018). Although advanced tertiary treatments at STPs are not yet regulated, SEPA is encouraging and supporting municipalities to introduce these processes. In this regard, Switzerland is the first country in the world with a

legal requirement for advanced tertiary treatments. This act was introduced in January 2016 with the aim to reduce micropollutant loads in effluents from the largest Swiss STPs (Bourgin et al., 2017). Tertiary treatment steps for micropollutants will be implemented until 2040 and so far, a few full-scale plants, mainly ozonation and/granular activated carbon steps have been implemented.

AOPs have proven to be effective in STPs for removal of micropollutants. The most studied advanced chemical oxidation process for the degradation of pharmaceuticals is ozonation (Lee et al., 2013) and it often used in combination with filtration through activated carbon for additional removal and an increased degree of purification. Ozone is a selective oxidant that removes many micropollutants. However, ozonation is ineffective against removing persistent micropollutants due to their chemical stability connected to their structural features, and thus show low reactivity. These micropollutants also often bioaccumulate, therefore when they are released into the environment, they can pose potential threats to aquatic and terrestrial organisms. To overcome the challenges posed by chemically stable substances, ozonation can be upgraded to a modified oxidation process called E-peroxone. The E-peroxone process involves the electrochemical conversion of oxygen (O_2) to hydrogen peroxide (H_2O_2), while generating highly reactive hydroxyl radicals (•OH) and hydrogen peroxide (H_2O_2) intermediates (Li et al., 2015). E-peroxone has been shown to be effective in removing ozone-resistant (O_3-resistent) micropollutants as well as those are easily removed by ozonation (Wang et al., 2019). The E-peroxone process also inhibits bromate formation, a carcinogenic compound, which is a major problem with ozonation (Von Gunten & Hoigné 1994, Wu et al., 2019). A major advantage is that ozonation can easily be retrofitted to E-peroxone since only air and electricity are used for this process. So far, E-peroxone has been proven to effectively remove micropollutants including pharmaceuticals as presented by Mustafa (2020).

2 INTRODUCTION

2.1 Strömsund sewage treatment plant

A summary of the main STP at Strömsund municipality is presented below. An overview of the process is also shown in Figure 1 and as appendix 2.

Strömsund municipal STP uses combined processes of mechanical, biological and chemical treatment steps for wastewater collected from Strömsund municipality. The plant is dimensioned for a hydraulic load (Q_{dim}) of 150 m^3/h and for a pollutant load corresponding to 7000 pe (population equivalents). The STPs design can mechanically process up to 600 m^3/h (4 Q_{dim}) while both the biological and chemical treatments have a maximum capacity of 300 m^3/h (2 Q_{dim}). A process scheme for Strömsund STP is shown in figure 1 and can also been found in appendix 2.

Incoming wastewater is funneled to an inlet pump station for further active transport for further treatment. The first mechanical treatment step is passing a strainer (3.0 mm mesh) followed by an aerated sand filter. Excess solids from the mechanical step are cleaned, dewatered and transported to Lidens recycling station where handled as combustible waste.

The biological treatment step is a Moving Bed Biofilm Reactor (MBBR-process) consisting of two sequential enclosed ponds with 160 m^3 volume each. The ponds are filled with suspending plastic carriers who facilitates growth of biofilm on its surfaces. The microorganisms who proliferates on the large combined surface area of the carriers can degrade organic material matter dissolved in the waste water, thus contribute to efficient removal of various agents to the continuously generated sludge.

Following the biological treatment is the chemical treatment. In a flocking chamber, aluminum-based chemicals are added to the sewage water for efficient precipitation of e.g., phosphorous. The water from the flocking chamber is lead to a sedimentation pond where the flocks generated are gravimetrically separated to the bottom of the pond as a chemical sludge.

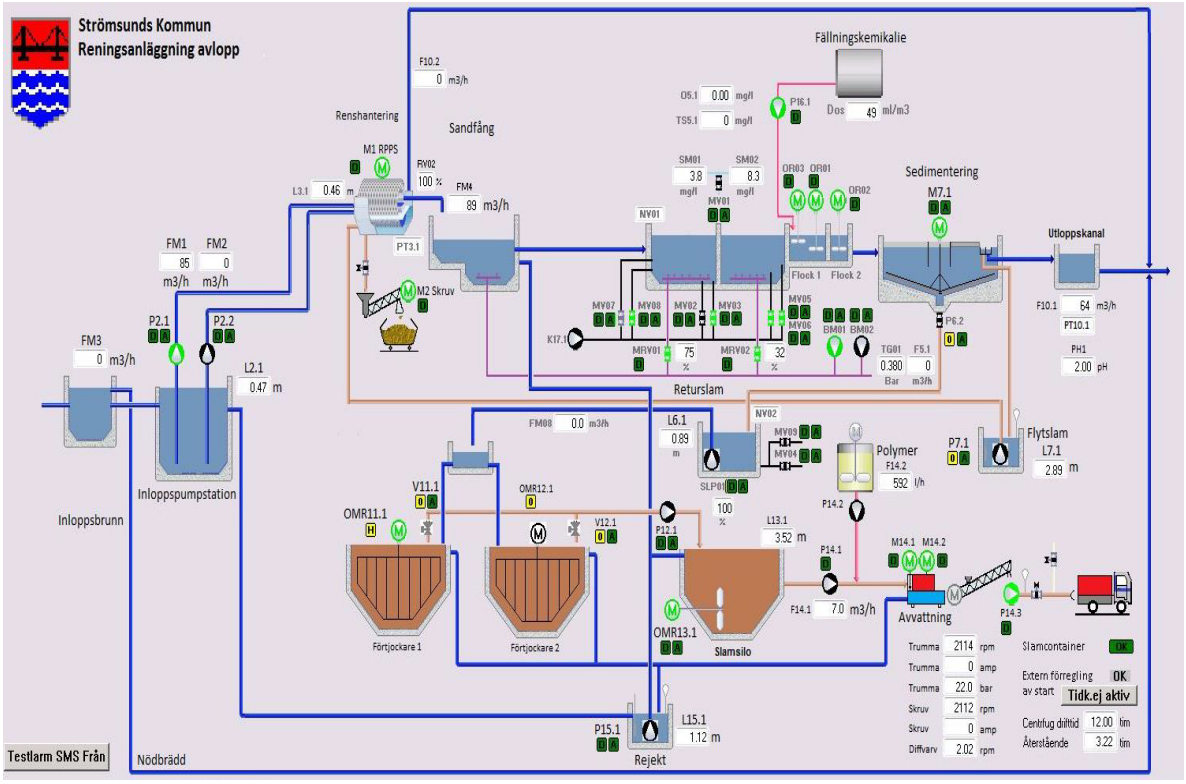

Figure 1. Process overview of Strömsund sewage treatment plant.

In the sedimentation both biological and chemical sludge are separated and treated water is continuously led to an outlet channel equipped with automated flow monitor and sampling station. The effluent passively run off to the recipient, lake Ströms Vattudal.

Sludge management contains a gravimetrical thickener before collected to a sludge chamber and dewatering in a centrifuge. Dewatered sludge goes to a closed container for further transport to a waste treatment facility where it finally is freeze-dried and composted with wood chips. Reject water from the thickener, sludge deposit, and centrifuge are recirculated back to the aerated sand filter.

2.2 Site orientation and recipient

As shown in Figure 2, Strömsund STP is situated in the central part of the municipality of Strömsund in the vicinity of Lake Ströms Vattudal. Strömsund STP is the largest treatment plant in the municipality and has recently (2017) been rebuilt and updated with a biological process (MBBR), see previous process description. By its size Strömsund STP, the quantity of pharmaceuticals is judged to be the largest in the municipality. The main recipient is Lake Ströms Vattudal (VISS-Water ID WA69022735 / SE708032-149042).

Good chemical surface water status prevails in the water body except for mercury (Hg) and polybrominated diphenyl ethers (PBDEs) which are a consequence of atmospheric deposition and not caused by a nearby source. Analysis of drugs and drug residues is missing for wastewater and recipient but have been conducted in this pilot project, see results section.

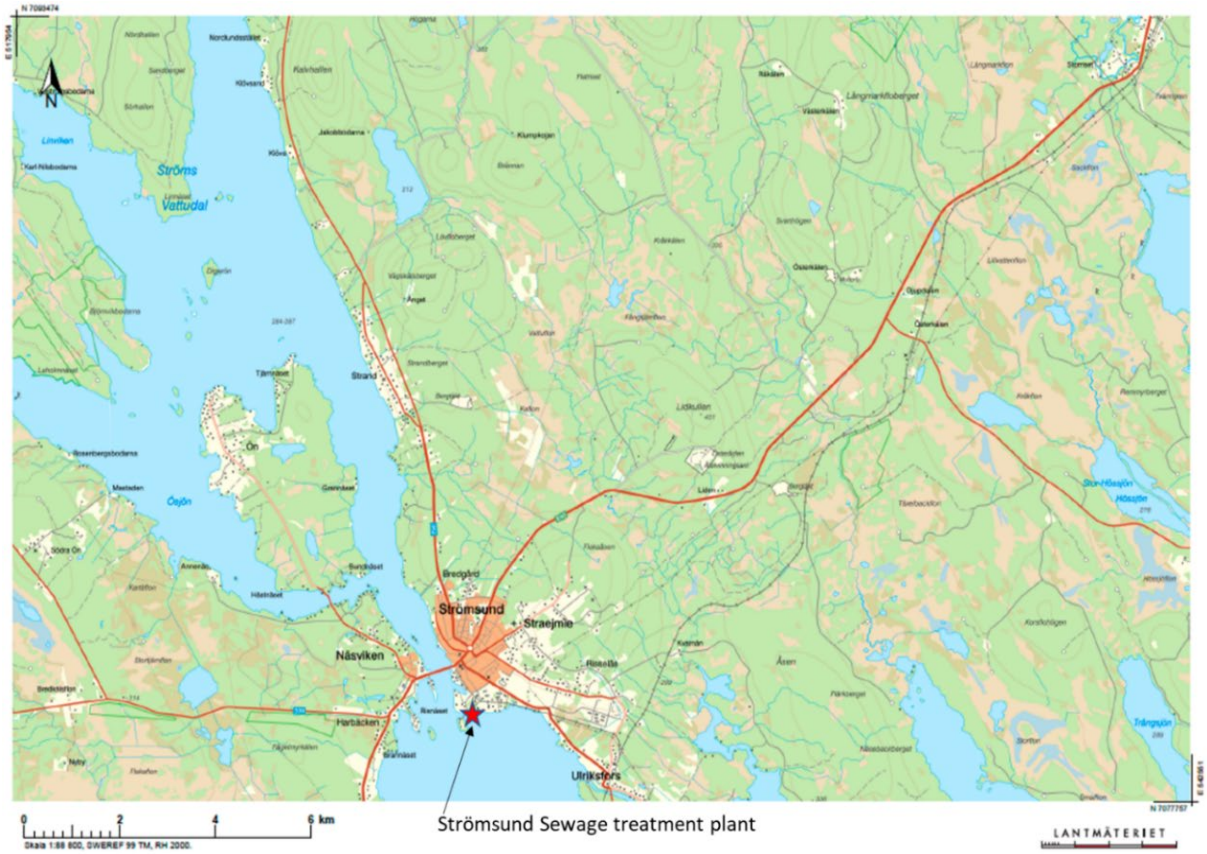

Figure 2. Location of Strömsund Sewage treatment plant

Currently, Lake Ströms Vattudal has a poor ecological status and constitutes a so-called heavily modified water (KMV). The lake system has been significantly affected in its hydrological regime or morphological state due to hydropower expansion. The quality requirement for the water body is moderate ecological potential. This means less strict quality criteria compared to unmodified water bodies, however viable aquatic life and populations must be able to be sustained within the water body. The Lake system of Ströms Vattudal is popular for sport fishing for stream-dwelling and migratory species of mainly brown trout and grayling and for pike and perch. The lake is very important for recreation, i.e., for fishing and other outdoor activities for people in the municipality. Among the requirements that must be met within the framework of ecological status is that aquatic species should be able to migrate freely within the lake systems and to possible tributaries and have sufficient access to spawning grounds. Implementation of new diverted water ways around dams has recently been implemented in the system with aim to increase fish migration within the system for improved ecological status and living conditions for migratory fish. The wild stock of brown trout (*salmo trutta*) is a rare strain (Bågedetrout) which grows especially large in the system with specimens reaching over 10 kg. The wild brown trout population in the lake has a very high ecological value and which today is threatened with extinction due to limitation in its migration and access to viable spawning grounds due to hydropower dams. Today, brown trout, grayling and other species generally have limited stream environments within the lake system because of the hydropower exploitment. The stocks can therefore be considered weakened and should preferably not be subjected to further stress, e.g., impact of drug residues.

The flow in the outlet of Ströms Vattudal which is part of the large river Faxälven is on average approx. 151 m^3/s as compared to the average flow at Strömsund's treatment plant which is around 1500 m^3/day. Protecting Ströms Vattudal is generally important for a sustainable water cycle and for the safety of environment and health in longer time perspectives. The

southernmost part of Ströms Vattudal consists of a Russfjärden which is the main catchment area and primary recipient for wastewater effluents from Strömsund STP. It has a lower mixing volume on a local scale compared to the northern parts of the lake with significantly larger water volume and average depths.

2.3 The project description

This project, financed by Swedish Enviromental Protection Agency (SEPA, Naturvårdsverket), is an up-scaled demonstration project of E-peroxone process for removal of pharmaceuticals and emerging contaminants in authentic sewage wastewater in Strömsund, Jämtland county, Sweden. It is together with a twin project in Lycksele, county of Västerbotten, Sweden, the first pilot project of its kind and to our knowledge, it is the first time this novel process has been implented at this scale. The study has been conducted using a capacity on industrial scale and constitutes a bridge between pilot and full scale and included the construction of a new treatment plant with a capacity of up to approx. 215 m^3/day.

E-peroxone is an ozone-based process and has the same basis for scaling up the technology. During this project, relevant comparison towards treatments using E-peroxone and only ozone has been performed to detect important differenses both in terms of removal efficiency of pharmaceuticals and regarding removal of toxicity. Overall, Strömsund demonstration project is also in line towards reaching United Nations global goals for sustainable development in Agenda 2030: Oceans and marine resources, Ecosystems and biological diversity, Clean water and sanitation for all, Sustainable cities and communities, Sustainable consumption and production. It is also well fitted for contributing to corresponding Swedish environmental goals in line with Swedish regulation (2018:495). Proposed analyses for evaluation of the project include analyses and parameters that all contribute to being able to meet the above-mentioned environmental goals.

3 AIMS OF THE PROJECT

The current pre-study had several objectives and aims which are listed below:

- Evaluate treatment efficiency of a novel advanced oxidation process, E-peroxone, in terms of removal of pharmaceuticals and other emerging contaminants.
- Achieve continuous treatment of pharmaceuticals and benzotriazoles from all waters that passes through Strömsund STP during different seasons.
- Compare E-peroxone process towards a more common AOP namely ozonation for treatment of STP effluent. Comparison to be made both for levels of pharmaceutical residues and change in ecotoxicological outcome using advanced analytical tools.
- Discuss results for full scale applications regarding costs, effectiveness and implication for environmental quality and human health.

4 SELECTION OF PHARMACEUTICALS TO BE STUDIED IN THIS PROJECT

According to Boxall et al (2012), more than 4000 pharmaceuticals are sold and used worldwide. In Sweden, more than 1200 pharmaceuticals were available on the Swedish market according to statistics from Apoteket AB, Sweden, in 2005. Among these 1200 pharmaceuticals that potentially can occur in aquatic systems, 98 pharmaceuticals of top priority were screened in this project. The selection process and the basis for choosing these 98 pharmaceuticals has been described thoroughly elsewhere (Fick et al., 2010; Grabic et al., 2012; Mustafa, 2020). Briefly describing, these 98 pharmaceuticals were prioritized based on their critical environmental concentrations and predicted environmental concentrations from amounts sold in Sweden in relation to commercially available standards for the analysis. So, they are top priority pharmaceuticals in terms of potentially adverse effects on fishes and amphibians that can be analysed. The list of screened 98 pharmaceuticals is provided in Table 1. In addition to pharmaceuticals, two triazoles that contains biocidal properties, were also screened which is another emerging class of micropollutants that have received attention in recent years due to environmental concerns (Östman et al., 2017). Another motivation to include these triazoles was the spread of Covid-19 that resulted in intensive use of difference disinfecting chemicals comprising biocides. Together, pharmaceuticals and triazoles will be referred as micropollutants hereinafter. In general, the screened micropollutants in this project are structurally diverse compounds and belong to different classes.

Table 1. Screened micropollutants in Lycksele sewage treatment plant effluent with their CAS numbers, limit of quantifications (LOQs) and class.

Compound	**CAS number**	**LOQ (ng L^{-1})**	**Class/Use**
Alfuzosin	81403-80-7	4	Urological
Alprazolam	28981-97-7	20	Psycholeptic
Amiodarone	1951-25-3	30	Antiarrhythmic drug
Amitriptyline	50-48-6	10	Antidepressant
Atenolol	29122-68-7	15	Hypertension drug
Atorvastatin	134523-00-5	10	Statin
Atracurium	64228-81-5	4	Muscle relaxant
Azelastine	58581-89-8	2	Anti-histamine
Azithromycin	83905-01-5	40	Antibiotic
Benzotriazole	95-14-7	50	Biocidal effect
Biperiden	514-65-8	3	Anti-Parkinson
Bisoprolol	66722-44-9	3	Hypertension drug
Bromocriptine	25614-03-3	15	Anti-Parkinson
Budesonide	51333-22-3	20	Anti-inflammatory corticoid
Buprenorphine	52485-79-7	20	Analgesic
Bupropion	34911-55-2	3	Antidepressant
Caffeine	58-08-2	20	Psycholeptic
Carbamazepine	298-46-4	7.5	Antiepileptic
Ceterizine	83881-51-0	15	Second-generation antihistamine
Chlorpromazine	50-53-3	10	Antipsychotic
Chlorprothixene	113-59-7	10	Antipsychotic
Cilazapril	88768-40-5	2	Hypertension drug
Ciprofloxacin	85721-33-1	10	Antibiotic
Citalopram	59729-33-8	15	Antidepressant
Clarithromycin	81103-11-9	3	Antibiotic
Clemastine	15686-51-8	2	Antidepressant

Clindamycin	18323-44-9	3	Antibiotic
Clomipramine	303-49-1	2	Antidepressant
Clonazepam	1622-61-3	10	Psycholeptic
Clotrimazol	23593-75-1	10	Antimycotic
Codeine	76-57-3	15	Analgesic
Cyproheptadine	129-03-3	7.5	Antihistamine
Desloratadine	100643-71-8	15	Antihistamine
Diclofenac	15307-79-6	10	Nonsteroid anti-inflammatory drug
Dicycloverine	77-19-0	10	Gastrointestinal disorder drug
Dihydroergotamine	511-12-6	15	Analgesic
Diltiazem	42399-41-7	2	Hypertension drug
Diphenhydramine	58-73-1	4	Antihistamine
Dipyridamole	58-32-2	3	Antithrombotic agent
Donepezil	120014-06-4	7.5	Anti-Alzheimer
Duloxetine	116539-59-4	2	Antidepressant
Eprosartan	133040-01-4	15	Hypertension
Erythromycin	114-07-8	20	Antibiotic
Felodipine	72509-76-3	20	Calcium channel blocker
Fenofibrate	49562-28-9	20	To treat hypercholesterolemia
Fexofenadine	83799-24-0	10	Antihistamine
Finasteride	98319-26-7	20	Urological
Flecainide	54143-55-4	2	Antiarrhythmic
Fluconazole	86386-73-4	7.5	Antimycotic
Flunitrazepam	1622-62-4	10	Psycholeptic
Fluoxetine	54910-89-3	7.5	Antidepressant
Flupentixol	2709-56-0	10	Psycholeptic
Fluphenazine	69-23-8	10	Psycholeptic
Glibenclamide	10238-21-8	20	Antidiabetic
Glimepiride	93479-97-1	20	Antidiabetic
Haloperidol	52-86-8	3	Psycholeptic
Hydroxyzine	68-88-2	3	Psycholeptic
Irbesartan	138402-11-6	3	Hypertension drug
Ketoconazole	65277-42-1	45	Antiandrogen
Levomepromazine	60-99-1	20	Psycholeptic
Loperamide	53179-11-6	2	Antipropulsive
Losartan	114798-26-4	10	Hypertension
Maprotiline	10262-69-8	15	Antidepressant
Meclizine	569-65-3	10	Antihistamine
Memantine	19982-08-2	3	Psycholeptic
Methyl-benzotriazole	136-85-6	50	Biocidal effect
Metoprolol	37350-58-6	15	Hypertension drug
Metronidazole	443-48-1	4	Antibiotic
Mianserin	24219-97-4	3	Antidepressant
Miconazole	22916-47-8	10	Antifungal
Mirtazapine	61337-67-5	15	Antidepressant
Naloxone	465-65-6	2	Opoid overdose drug-Narcotic antagonist
Nefazodone	83366-66-9	2	Antidepressant
Norfloxacin	70458-96-7	20	Antibiotic
Ofloxacin	82419-36-1	3	Antibiotic
Orphenadrine	83-98-7	3	Antihistamine
Oxazepam	604-75-1	10	Psycholeptic
Paracetamol	103-90-2	30	Analgesic

Paroxetine	61869-08-7	10	Antidepressant
Perphenazine	58-39-9	20	Psycholeptic
Pizotifen	15574-96-6	2	Analgesic
Promethazine	60-87-7	15	Neuroleptic
Propranolol	525-66-6	20	Beta blocking agent
Ranitidine	66357-35-5	20	Peptic ulcer drug
Repaglinide	135062-02-1	2	Antidiabetic
Risperidone	106266-06-2	4	Psycholeptic
Rosuvastatin	287714-41-4	20	Statin
Roxithromycin	80214-83-1	15	Antibiotic
Sertraline	79617-96-2	10	Antidepressant
Sotalol	3930-20-9	15	Hypertension drug
Sulfamethoxazole	723-46-6	15	Antibiotic
Tamoxifen	10540-29-1	5	Estrogen receptor modulator
Telmisartan	144701-48-4	10	Hypertension drug
Terbutaline	23031-25-6	3	Broncodilator
Tramadol	27203-92-5	15	Analgesic
Trihexyphenidyl	144-11-6	3	Anti-Parkinson
Trimethoprim	738-70-5	3	Antibiotic
Venlafaxine	93413-69-5	20	Antidepressant
Verapamil	52-53-9	10	Hypertension drug
Zolpidem	82626-48-0	3	Psycholeptic

5 THE CONSTRUCTION AND TESTING OF PILOT SYSTEM

The pilot plant with E-peroxone and ozonation processes was built in a 20-foot insulated mobile shipping container at Envix's industrial hall in Umeå. The ready pilot was shipped to Strömsund and installed in the water treatment industrial hall at Strömsund municipal STP as shown in Figure 3. The electrochemical cell for in situ generation of hydrogen peroxide and the treatment tank were installed outside of the container.

For ozone production, pressure swing adsorption (PSA) oxygen generating system was used to provide high purity oxygen with >90% purity. The pressure and flow of oxygen was regulated for optimal performance of ozone generator to generate ozone. The ozone production was regulated by the production power of the ozone generator and the concentration of produced ozone was continuously monitored by ozone analyzer. Gaseous ozone was injected into the wastewater via two injections by splitting them, i.e., one via venturi and the other via diffusers installed in the bottom of reaction chamber. Residual ozone gas from the reaction chamber was sent to the ozone destruct unit to convert it back to oxygen which was further venting-off. For E-peroxone treatment, an electrochemical cell, consisting of multiple cathodes and anodes, was continuously operating for in situ generation of hydrogen peroxide, and was providing required dose of hydrogen peroxide.

The water line was equipped with all necessary flow and pressure meters. All the electrical components were connected to the central PLC in the pilot with the option to operate and monitor the system via PLC screen in the container as well as remotely. All necessary safety sensors e.g., ambient ozone level, ambient oxygen level, water leaks etc. were connected to PLC system for self-control of the system in case of any leaks.

The incoming (feed) water for the AOP pilot was taken from the sencondary effluent (outgoing water) of Strömsund STP. The incoming water flow rate for the AOP pilot system was set at 9 m^3/h, corresponding to approx. 15 % of the average flow of Strömsund STP, with a total

hydraulic retention time of 30 minutes for AOP treatment unit. Strömsund STP provided an electricity connection point which was providing electricity to the mother electrical panel of the AOP pilot.

As a first step, ozonation process was optimized by optimizing ozone dose for micropollutants removal. For E-peroxone process, H_2O_2 dose was optimized in relation to optimal ozone dose by testing these doses at different ratios. The samples were collected before and after treatment of AOP processes to understand micropollutants removal as well as toxicity removal of the treated water. The same optimized parameters were used to run the the E-peroxone process in different seasons.

Figure 3. Photo of E-peroxone pilot system at Strömsund sewage treatment plant.

5.1 Testing period and sampling

The continuous test run of AOP pilot was conducted during four different seasons i.e., winter, autumn, summer and spring, for two weeks each season (except for spring testing) and over the period of one year (i.e., 2022). The winter testing was conducted during November 14-25, autumn testing during September 12-23, summer testing during July 18-27, and spring testing during April 4-May 19, 2022. The spring testing lasted longer as the pilot optimization was conducted during the same period. All the collected samples of these test runs were prepared on site immediately after collection and sent the for micropollutants and toxicity analysis.

5.2 Analytical methods

5.2.1 Analysis of pharmaceuticals

For detection of target micropollutants in the water, 10 mL samples were collected. Each sample was filtered using a 45 µm syringe filter. To follow potential losses of micropollutants, 40 and 68 µL of pharmaceuticals and biocides internal standards (ISs) were added to each sample, respectively. The detail of ISs is provided elsewhere (Grabic et al., 2012; Lindberg et al., 2014; Östman et al., 2017). For AOP treated sample, 100 µL of sodium thiosulfate (0.1 M) was immediately added to each sample after collection to quench the residual oxidants. Samples were stored at 4 °C in the dark and analyzed at department of Chemistry, Umeå University within 2 weeks of collection. The micropollutants were analyzed as reported previously (Grabic et al., 2012; Lindberg et al., 2014; Mustafa., 2020).

Briefly, automated online solid phase extraction (SPE) was used in combination with liquid chromatography (LC) and triple stage quadrupole mass spectrometry (MS/MS). Samples were acidified to pH 3 using formic acid and 1 mL of the resulting solution was injected into the LC-MS/MS system (Thermo Fisher Scientific, San Jose, CA, USA) via a 1 mL loop. Injected samples were then passed through an OASIS HLB (20 mm × 2.1 mm i.d., 15 µm particle size) online extraction column (Waters, Milford, Massachusetts, USA) followed by a guard column (20 mm × 2.1 mm i.d.) and an analytical column (50 mm × 2.1 mm i.d.). Both the guard and analytical columns were supplied by Thermo Fisher Scientific (San Jose, CA, USA) and contained 5 µm particles of Hypersil GOLD aQ C18 polar end-capped stationary phase. The analytes were ionized by heated electrospray ionization (HESI), in negative or positive ion mode. Vaporizer and capillary temperatures were 200 °C and 325 °C, respectively and the ionization voltage was 3.5 kV. Argon was used as the collision gas at a pressure of 1.5 mTorr and the resolution of the mass analyzing quadrupoles was 0.7 FMWH. Micropollutants were quantified by internal standard calibration (ISC) (see SI Table S1 for the LOQs). The total time required for a complete analysis (online extraction and LC-MS/MS analysis of a sample) was approximately 15 minutes.

5.2.2 Toxicity analysis

Within this project, focus has mainly been on investigating the removal efficiency of micropollutants by the descriped E-peroxone process. However, one part of the evaluation is to reflect to treatment outcome using effect measurement in ecotoxicologically relevant testing models. Measuring diverse effects from pharmaceuticals is not an easy task within ecotoxicology. To reflect toxicity in this project, we have used both mortality/immobilisation in the fresh water living crustacean *Daphnia magna*. The *Daphnia sp*. Acute Immobilisation test is based on the OECD test 202 and the results were presented as the concentration of the test media where 50% inhibition of mobility occurs (IC50).

Daphnia magna is among the most sensitive organisms when compared to other species within the taxonomy within ecotoxicology. When effect levels are compared in species sensitivity distributions (SSDs), Daphnids sp. are often in the lower range of measured effect levels and stipualted PNECs (predicted no effect concentrations). Therefore , *Daphnia Magn*a is a suitable model organisms to study in samples expected to contain target contaminants in the ng/L range.

Sumpter et al (2022) acknowledged that little work has been done within characterizing the effects from pharmaceuticals at environmentally relevant concentrations and how prioritization should be done among the vast variety of pharmaceuticals, and which ones pose the potentially largest threats to the environment and human health. Sumpter et al (2022) stressed that in using bioassays and markers reflecting sublethal effects and the initiation of molecular events could be

very useful in trying to understand the impact from combined exposure of pharmaceutical residues in surface waters.

In addition to measuring mortality in *Daphnia magna*, reproductive and toxicological gene expressions was studied following exposure to a selection of water samples in this project. This methodology has also recently been accepted as SIS-CEN technical specification (SIS-CEN/TS 17883:2022). Studying gene expressions on the mRNA level for a selection of toxicologically relevant marker genes presumably can give valuable insight on the mechanistic and causal relationship to a certain exposure. With knowledge on the purpose of different genes and their functionality for various physiological processes, causal-relationships can potentially be explored during testing.

Besides using *Daphnia magna*, three different isolated human cell lines were also used for studying other types of effects by measuring differences in gene expressions. HepG2 cells were used for studying the expression of cytochrome P450 1A1 (Cyp1A1) and the aryl hydrocarbon receptor (AhR). These genes will reflect exposure to dioxin and dioxin like organics substances as well as different polyaromatic hydrocarbons (PAHs). Further, several genes reflecting the occurrence of oxidative stress, free radicals and organic metabolites were tested by analyzing gene regulation of Glutatione-S-transferase (Gst), catalase (Cat) and superoxide dismutase (Sod1, 2 and 3). In addition, also metal exposure and oxidative stress were indicated by analysing the regulation of metallothioneine MT1A in the HepG2 cells. HepG2 cells were isolated from the liver and is suitable for toxicity testing since the liver is the principal site for metabolism and biotransformation of xenobiotics in phase I and phase II reactions.

MCF7 cells were used in another bioassay as part of the evaluation of the effluent. MCF7 are originally isolated from breast cancer and respond to estrogenic compounds. Therefore, the gene expressions of estrogen receptors alfa (Era) and beta (Erb) were analyzed in these cell experiments. Besides Era and Erb , the Cyp1A1 gene was studied in MCF7 cells.

The analysis in THP1 cells were used for reflecting functionality of the immune system after exposure to untreated and treated effluent samples. THP1 cells are monocytes and were originally isolated from a leukemia patient and is commonly used for studying immune system disorders. To detect effects on the immune system, the analysis in THP1 cells was done on protein level by measuring interleukin 6 (IL-6) and tumor necrosis factor -alfa (TNF-alfa). Both proteins are very important for the function of the immune system, e.g., in the initiation of inflammatory response and for the identification of antigens.

6 RESULTS

6.1 Characterization of Strömsund STP effluent before and after E-peroxone

Lycksele STP effluent was characetrized and compared with the treated effluent by E-peroxone process and ozonation. The wastewater characterization results are presented in Table 2. The pH and the total organic carbon (TOC) of the secondary effluent remained unchanged before and after E-peroxone and ozonation treatments. A slight change in dissolved organic carbon (DOC) was observed after both processes. For example DOC was increased to 9.5 from 9 mg/L after ozonation treatment whereas slightly decreased to 8.8 after E-peroxone treatment.

The color of the effluent decreased after both treatments and this can be attributed to the oxidation of organic matter as well as some colour giving compounds. The turbidity of the effluent remained unchanged after ozonation however, increased almost three times after E-peroxone. Alkalinity of the effluent also decreased 38% by E-peroxone and 34% by ozonation.

The chemical oxygen demand (COD)of the effluent remained same whereas biochemical oxygen demand (BOD) increased by 33% after ozonation. In contrast, COD of the effluent treated by E-peroxone decreased 11% while COD remained unchanged. Phosphorus level in the secondary effluent decreased by 40% after ozonation while remained unchanged after E-peroxone process. Both oxidation processes had no effect on the total nitrogen present in the effluent. In general, both E-peroxone and ozonation showed almost similar effect on most of the basic water characterics.

Table 2. Water characterization parameters of Strömsund STP effluent and treated effluent after ozonation and E-peroxone.

Parameter	***Unit***	***Secondary effluent from Strömsund STP***	***Treated secondary effluent after ozonation***	***Treated secondary effluent after E-peroxone***
pH		7.7	7.5	7.7
Color (410 nm)	mg Pt/L	23	<5,0	<5,0
Turbidity	FNU	2.4	2.7	6.8
Alkalinity	mg HCO_3/L	290	190	180
Dissolved organic carbon (DOC)	mg/L	9	9.5	8.8
Total organic carbon (TOC)	mg/L	11	11	11
Chemical oxygen demand (COD-Cr)	mg/L	28	29	25
Biochemical oxygen demand (BOD)	mg/L	6	8	6
Phosphorus (P)	mg/L	0.2	0.12	0.21
Total nitrogen	mg/L	20	21	21

6.2 Occurrence of micropollutants at Strömsund STP

In total, 100 micropollutants were screened during four seasons in Strömsund STP effluent. Every season, approx. 2 samples were analysed with screening of all 100 pharmaceuticals. Out of 100, 43 micropollutants were detected in the effluent. The average of detected micropollutants concentrations during four seasons is shown in Figure 4. The most common pharmaceuticals detected in the effluent were belonging to hypertension and antidepressant classes (7 pharmaceuticals in each class) of pharmaceuticals following by antibiotics (6 pharmaceuticals). After them, the other detected pharmaceuticals were belonging to phycholeptic (4), analgesic (4), antihistamine (4) and statin (2). Both triazoles i.e., benzotriazole and methyl-benzotriazole were also found in the effluents. Out of 43, the detection frequency of 25 micropollutants was 100%, 10 micropollutants between 50-100% while 8 micropollutants had less than 50%.

The concentrations of detected micropollutants were varying over several order of magnitude from ng/L to µg/L. Stimulant caffeine was detected at highest average concentration i.e., 53 µg/L while lowest average concentration was found for azelastine i.e., 6 ng/L in Strömsund STP effluent. After caffeine, the hypertension drug metoprolol and analgesic and antipyretics drug paracetamol had the highest concentrations, ranging 3-4 µg/L. Interestingly, both triazoles i.e., benzotriazole and methyl-benzotrizole were detected at relatively high concentrations, i.e., 1018

ng/L and 744 ng/L, respectively. This is similar to the previous study where micropollutants were detected at 11 different STPs in Sweden (Strömsund not included) and benzotriazoles were the most common compounds in the effluents in the same concentration range i.e., 900 ng/L (Östman et al., 2017). These findings emphasize that other classes of potentially hazardous micropollutants should also be considered when upgrading STPs with tertiary treatment for removal of micropollutants.

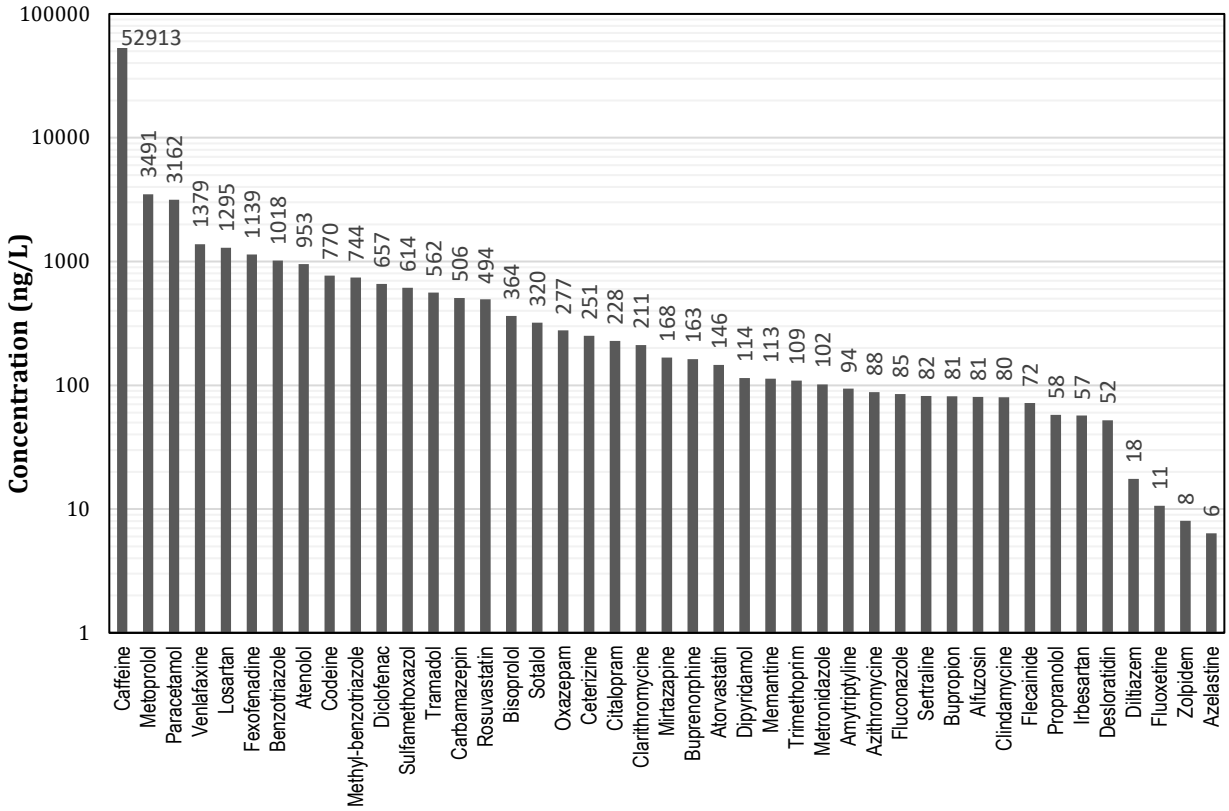

Figure 4. Occurrence of micropollutants in Strömsund Sewage treatment plant effluent.

Out of 43 detected micropollutants in Strömsund STP effluent, 12 marker micropollutants were studied intensively for this project (see Table 3), analyzing them after approx. every 48 hours during the two weeks of testing for all four seasons. The selection of these marker micropollutants was based on several factors such as varying structures, detection frequency, guidelines from Swedish or EU directives and most importantly varying reactivity of ozone with these micropollutants due to the selective nature of ozone. The minimum, maximum and average level of these compounds for all four seasons are presented in Table 3 with their detection frequency.

All marker micropollutants detected with 100% frequency except for paracetamol and methyl-benzotriazole. Paracetamol detection frequency varied between 30-100%. The lowest detection frequency was observed during summer season followed by winter while the highet detection frequency was seen for spring season. In connection to that, the highest detected concentration was also observed for spring season which varied from rest of the seasons. The detected concentrations during the three seasons i.e., winter, summer and autumn were similar. The detection frequency for methyl-benzotriazole was 100% except for autumn season i.e., 80% and the average concentration over the four seasons did not vary significantly. Substantial variation in average concentrations of diclofenac were observed for all four seasons. Interestingly, highest

Table 3. Concentrations of marker micropollutants at minimum, maximum and average levels (in ng/L) in Strömsund sewage treatment plant effluent during four seasons in 2022, along their detection frequency and second order rate constants for ozone (ko_3) and hydroxyl radicals ($k_{\bullet OH}$).

Marker Compounds	ko_3 ($M^{-1}s^{-1}$)	$k_{\bullet OH}$ ($M^{-1}s^{-1}$)	Winter Season (14-25 Nov 2022)					Autumn Season (12-23 Sep 2022)					Summer Season (18-27 July 2022)					Spring Season (4 April – 19 May 2022)				
			Min.	*Max.*	*Avg.*	*Std. Dev.*	*Det. fre. (%)*	*Min.*	*Max.*	*Avg.*	*Std. Dev.*	*Det. fre. (%)*	*Min.*	*Max.*	*Avg.*	*Std. Dev.*	*Det. fre. (%)*	*Min.*	*Max.*	*Avg.*	*Std. Dev.*	*Det. fre. (%)*
Benzotriazole	240 [a]	7.6×10^{9} [a]	374	1221	717	344	100	1283	2359	1605	431	100	239	1927	869	731	100	559	1317	880	360	100
Carbamazepine	3×10^{5} [b]	8.8×10^{9} [b]	204	506	328	110	100	641	987	784	152	100	397	581	484	62	100	267	595	432	181	100
Citalopram	1.1×10^{3} [c]		149	390	293	101	100	201	396	289	82	100	92	275	143	68	100	88	233	187	67	100
Diclofenac	1×10^{6} [b]	7.5×10^{9} [b]	59	624	257	213	100	808	2765	1468	751	100	<LOQ	543	347	175	70	173	1006	557	343	100
Fluconazole	<1 [a]	4.6×10^{9} [a]	32	203	98	57	100	62	163	99	35	100	38	107	70	25	100	59	89	74	13	100
Irbesartan	24 [d]	10^{10} [d]	10	54	26	16	100	41	105	77	24	100	37	159	93	55	100	12	46	31	15	100
Memantine	7.75 [c]		54	141	117	35	100	110	204	164	39	100	30	90	64	24	100	79	148	109	30	100
Methyl-benzotriazole	780 [a]	8.6×10^{9} [a]	327	1282	766	351	100	<LOQ	885	507	254	80	650	961	858	109	100	291	2260	845	946	100
Metoprolol	2.0×10^{3} [a]	7.3×10^{9} [e]	2550	3612	3005	475	100	4116	7102	5584	1168	100	1559	1950	1720	176	100	2785	4658	3657	792	100
Oxazepam	~1 [a]	9.1×10^{9} [a]	136	298	201	65	100	380	535	462	66	100	134	319	189	67	100	206	345	254	65	100
Paracetamol	2.57×10^{6} [f]		<LOQ	3031	2772	367	30	ND	4478	2625	1599	70	<LOQ	2252	2252	–	20	1128	7412	5000	2704	100
Trimethoprim	2.7×10^{5} [a]	6.9×10^{9} [g]	57	138	94	33	100	65	260	152	83	100	14	84	45	26	100	48	333	145	128	100

<LOQ: Below limit of quantification

[a] (Lee et al., 2014), [b] (Huber et al., 2003), [c] (Mustafa et al., 2021), [d] (Bourgin et al., 2017), [e] (Benner et al., 2008), [f] (Hamdi El Najjar et al., 2014), [g] (Dodd et al., 2006)

average concentrations of diclofenac, carbamazepine, benzotriazole and metoprolol were seen during the autumn season. Among them, least variation in average concentrations during all the seasons was observed for antimycotic fluconazole. The overall dectection frequency of marker compounds are significantly lower in Strömsund STP effluent as compared to Lycksele STP effluent.

6.3 Mass flow of micropollutants

The total mass flow of the micropollutants per day, based on 100 screened compounds, were calculated in Strömsund STP effluent for each season and presented in Table 4. Variations in mass flows can be seen for different seasons. The largest mass flow of 0.17 kg/day was observed for spring season followed by autumn season with 0.11 kg/day. The lowest mass flow was found for summer season indicating overall less use of pharmaceuticals during summer. Note that the daily mass flow is representing average daily wastewater flow i.e., ~1500 m^3/day at Strömsund STP. The average mass flow of all four seasons indicates that 0.11 kg of these micropollutants (from 100 screened micropollutants) are being released every day by Strömsund STP into Lake Ströms Vattudal. By expanding the mass flow for the whole year with the same average flow, Strömsund STP is releasing ~40 kg of pharmaceuticals into the environment (Lake Ströms Vattudal) with its conventional treatment processes. It is worth mentioning that this number i.e., 40 kg/year is based on screening of 98 pharmaceuticals and 2 benzotriazoles. As mentioned in the previous section, approx. 1200 pharmaceuticals are sold in the Swedish market. Considering the same detection ratio of these 1200 pharmaceuticals, the actual pharmaceuticals mass releasing into the environment by Strömsund STP is expected to be much higher.

Table 4. Mass flow of micropollutants based on screening of 98 pharmaceuticals and 2 benzotriazoles from Strömsund sewage treatment plant into the environment.

Season	**Mass flow of micropollutants (kg/day)**
Winter season	0.09
Autumn Season	0.11
Summer Season	0.06
Spring Season	0.17
Average	0.11

6.4 Micropollutants removal during ozonation and optimization of ozone dose

For ozonation, Strömsund STP effluent was treated at three different ozone concentrations for micropollutants removal. These concentrations correspond to specific ozone doses (SODs) of 0.6 gO_3/gDOC, 0.9 gO_3/gDOC and 1.1 gO_3/gDOC. The removal of marker micropollutants at all three SODs is shown in Figure 5. As mentioned in earlier section, the marker compounds were diverse in terms of ozone reactivity, thus classified into three groups based on their reactivities with ozone (as second-order rate constants, k_{O3} ($M^{-1}s^{-1}$)), more specifically, O_3-reactive ($k_{O3} > 10^4$ $M^{-1}s^{-1}$), moderately O_3-reactive ($10^2 < k_{O3} < 10^4$ $M^{-1}s^{-1}$) and O_3-resistant ($k_{O3} < 10^2$ $M^{-1}s^{-1}$). Available second order rate constants of these marker compounds for ozone and hydroxyl radicals (•OH)

are provided in Table 3. The marker micropollutants in Figure 5 are arranged according to their k_{O3} values ($M^{-1}s^{-1}$) which are decreasing from left to right.

Removal of O_3-reactive micropollutants: All marker O_3-reactive micropollutants i.e. diclofenac, trimethoprim and carbamazepine were completely removed at a SOD of 0.6 gO_3/g DOC during ozonation except for paracetamol which removed 90%. The complete removal (or very high removal) of these micropollutants can be attributed to their high k_{O3} values ($k_{O3} > 10^4$ $M^{-1}s^{-1}$) (Lee et al., 2014). Since these micropollutants were either completely removed or showed very high removal at 0.6 g O_3/g DOC, further increase in SOD to 0.9 and 1.1 g O_3/g DOC did not affect the removal of these compounds. In general, these results agree with other studies that reported SODs of 0.5 gO_3/gDOC (Lee et al., 2014) and 0.47 gO_3/gDOC (Hollender et al., 2009) to remove >90% of O_3-reactive micropollutants. Very high removal of O_3-reactive micropollutants is attributed to electron rich (donating) moieties in these compounds which enable them for direct reaction with ozone. This is why, the direct reaction of ozone accounts for 82% of the removal of O_3-reactive micropoluutants while •OH mediated removal explaining the remaining 18% (Lee et al., 2013).

Removal of moderately O_3-reactive micropollutants: Of the four marker moderately O_3-reactive micropollutants ($10^2 < k_{O3} < 10^4$ $M^{-1}s^{-1}$), benzotriazole and methyl-benzotriazole belonged to emerging class of micropollutants i.e. biocides. Among this group, only methyl-benzotriazole was removed by >90% at 0.6 gO_3/gDOC because ozone attacks benzo moieties in benzotriazoles and cause direct oxidation (Mawhinney et al., 2012)), while the other three marker compounds showed incomplete removal ranging 36-78% at this ozone dose with lowest removal for benzotriazole. The relatively poor elimination of these micropollutants especially benzotriazole can be attributed to the fact that they have lower k_{O3} than O_3-reactive marker compounds. As such, Lee et al. (2013) reported that an additional source of •OH (Lee et al., 2013) or higher ozone dose is required for their complete removal especially for micropollutants with low k_{O3}.

Further, an increase in SOD to 0.9 gO_3/gDOC resulted in improved removal of citalopram and metoprolol. For instance, citalopram removed completely (100%) with an increased removal of 22% while metoprolol removed 87%, an increase of 15%. However, no improvement was seen for benzotriazole and still showed poor removal i.e., 32% at 0.9 gO_3/gDOC. Higher removal of methyl-benzotriazole as compared to benzotriazole is attributed to electron-donating methyl substituent which increases the electron density of the molecule and facilitates electrophilic ozone attack (Von Sonntag and von Gunten, 2012). Further increasing the ozone dose to 1.1 gO_3/gDOC resulted in improved removal of benzotriazole to 58%, a 26% increase from 0.9 gO_3/gDOC.

Removal of O_3-resistant micropollutants: All O_3-resistant micropollutants ($k_{O3} < 10^2$ $M^{-1}s^{-1}$) also showed poor removal at all three SODs except for irbesartan. Interestingly, irbesartan, which has been showing poor removal due to low ozone rate constant in previous study (Mustafa, 2020), removed completely at all SODs in this study. The reason for this behavior can be related to low concentration of detected irbesartan, i.e., 28 ng/L. Apparently, all SODs were sufficient to remove this much level of irbesartan. However, with single ozone injection reactor design, still small amount of residual irbesartan was detected from this low concentration (with approximately 65% removal) (the results of this reactor design is not presented in this report). So, another factor for complete removal along with low level of irbesartan could be ozonating wastewater using two injections reactor design.

The other three marker O_3-resistant micropollutants including memantine, oxazepam and fluconazole showed incomplete removal in the range of 38-63% at SOD of 0.6 gO_3/gDOC during ozonation. Increas in ozone dose to 0.9 gO_3/gDOC improved the removal of only fluconazole to 77% with an increase of 38%. However, no improvement in removal for memantine and oxazepam was observed. Further increasing the SOD to 1.1 gO_3/gDOC, no improvement in

removal of any micropollutant was observed. In fact, the removal for memantine decreased to 30% at 1.1 gO_3/gDOC from 62% at 0.9 g O_3/g DOC, decrease of 32%. O_3-resistant micropollutants are predominantly oxidized by •OH (Katsoyiannis et al., 2011) due to low ozone reactivity and are often only marginally removed from wastewater because the formation of •OH, which depends on the water matrix, is often slow in relation to hydraulic residence (Von Sonntag and von Gunten, 2012). In general, the removal of marker O_3-resistant micropollutants during ozonation is relatively slightly higher in this project than previous studies. The two possible reasons for this behavior could be two injections of ozone and the longer hydraulic residence time than those studies.

Due to optimal removal of micropollutants, SOD of 1.1 gO_3/gDOC was chosen for rest of the project and for optimization of E-peroxone process and testing during different seasons.

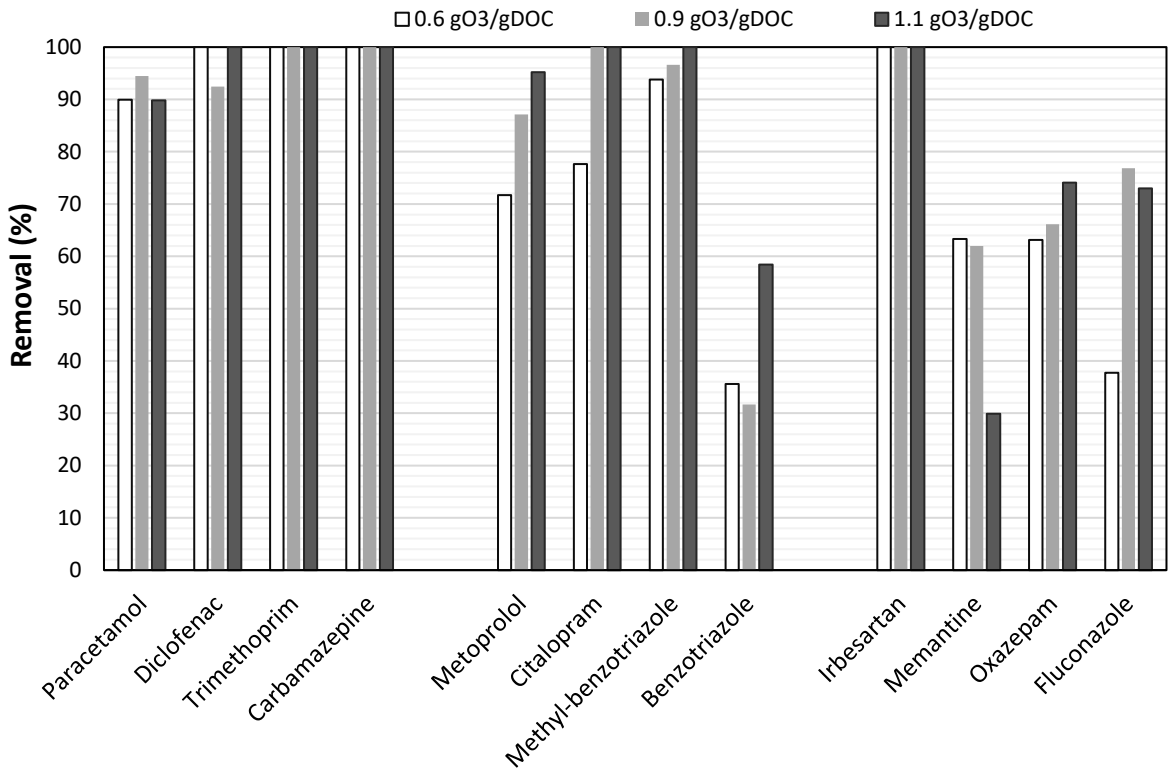

Figure 5. Removal (in %) of micropollutants in Strömsund wastewater effluent during ozonation with SODs of 0.6, 0.9 and 1.1 gO_3/gDOC.

6.5 Removal of micropollutants during E-peroxone in comparison to ozonation

Ozonation alone showed poor or incomplete removal for few moderately O_3-reactive and most of the O_3-resistant micropollutants at the tested ozone doses. Among them, 1.1 gO_3/gDOC appeared to be the optimal dose for removing micropollutants during ozonation. Thus, this ozone dose was used during the E-peroxone process and for the testing of E-peroxone during different seasons. The removal of marker micropollutants by the E-peroxone process in comparison to ozonation is illustrated in Figure 6. The ratio of ozone and hydrogen peroxide i.e., $[O_3]$:$[H_2O_2]$ was set between 0.5-0.6 for E-peroxone treatment of Strömsund STP effluent.

As expected, the E-peroxone process had no effect on the removal of O_3-reactive compounds. In fact, these micropollutants were supposed to be removed by ozonation with reactor design of injecting ozone in two injections. These micropollutants, with very high ozone reactivity, are removed quickly even at very low ozone dose and expected to remove at first injection of ozone

even before E-peroxone process starts. Thus, it can be concluded that the removal results of O_3-reactive micropollutants, presented in Figure 6, is presenting their removal by ozonation rather than E-peroxone process.

Further, E-peroxone process showed varying effects on the removal of micropollutants that had moderate reactivity with ozone. For instance, metoprolol, citalopram and methyl-benzotriazole removal decreased insignificantly (9%, 9% and 4%, respectively) by E-peroxone process, and yet giving high removal of 86%, 91% and 96%, respectively. The slight decrease in removal of moderately O_3-reactive micropollutants can be attributed to their relatively high second order rate constants. By switching to E-peroxone, the selective oxidation of these micropollutants by ozonation is slightly decreased (>10%) due to the side reaction of ozone with electrochemically generated hydrogen peroxide. On the other hand, removal of benzotriazole increased by 22%, giving a total removal of 80%. This is because benzotriazole has lowest ozone reactivity in terms of second order rate constant and thus reason for the low removal during ozonation. By switching to E-peroxone, the formation of $^{\bullet}OH$ is accelerated by the reaction of ozone and hydrogen peroxide. As a results, the oxidation (and removal) of benzotriazole increased.

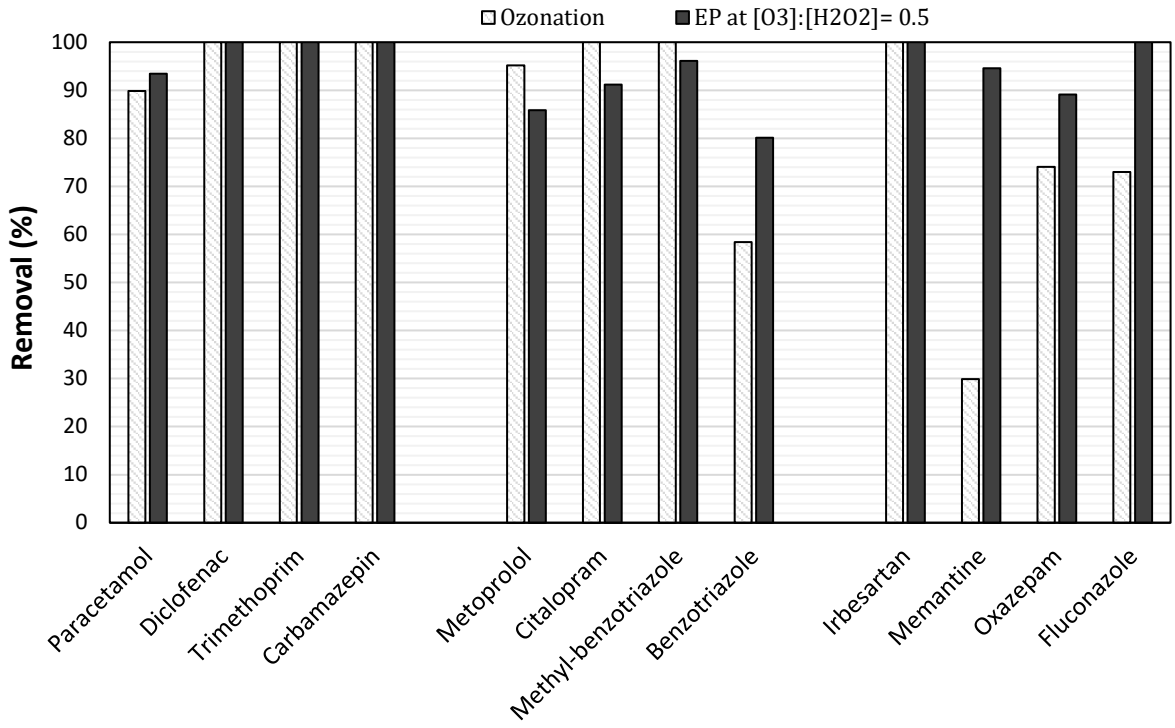

Figure 6. Removal (in %) of micropollutants in Strömsund secondary wastewater effluent during E-peroxone process at $[O_3]:[H_2O_2]=0.5$ in comparison to ozonation alone at 1.1 $gO_3/gDOC$.

The E-peroxone process improved the removal of all marker O_3-resistant micropollutants in comparison to ozonation except for irbesartan which was removed completely by bothe processes due to low concentrations in secondary effluent (see previous section for the discussion). The E-peroxone process was able to remove 95% of memantine, 89% of oxazepam, and complete removal (100%) of fluconazole, which represent improvements of 65% for memantine, 15% for oxazepam and 27% for fluconazole by E-peroxone process from ozonation. These results contradict with previous findings that suggest E-peroxone process only marginally improves (< 10%) the removal of micropollutant in wastewater when compared with ozonation (Yao et al., 2018). However, these results are in line with our previous work (Mustafa, 2020) and other studies (Cruz-Alcalde et al, 2020) which showed the same results of improved removal.

This discrepancy in results may be explained by use of appropriate ozone dose which is higher than instantaneous ozone demand required by the secondary wasetwater effluent. The residual ozone after instantaneous ozone demand of water matrix could then react with the electrochemically-generated H_2O_2 (for E-peroxone), potentially accelerating the formation of $^{\bullet}OH$ to improve the removal of micropollutants with low ozone reactivity (Cruz-Alcalde et al., 2020).

In general, it can be concluded that the current pilot system improved the removal of persistent micropollutants significantly both from ozone resistant and moderately ozone reactive groups. A slight insignificant decrease in removal of few micropollutants was seens for moderately ozone reactive group where no effect on micropollutant removal by E-peroxone was observed for ozone reactive micropollutants group by the pilot built in this project.

6.6 Removal of micropollutants over different seasons

The E-peroxone pilot system was tested with continuous run for three seasons and almost two weeks each season. The purpose of testing the system during different seasons was to test the robustness of the system in different seasons as well as understand the effect of variation in the water characteristics on micropollutants removal. The results of all four seasons are presented in Table 5 to 8. Moreover, at least one sample was analyzed in all seasons (except for spring season) to estimate the removal of all detected micropollutant based on screening of 100 micropollutants, and the results are presented in Table S1.

6.6.1 Winter Testing

The winter testing was conducted during the period of 14/11/2022 to 25/11/2022 with the continuous run of the pilot system. The treated water samples were collected after almost every 48 hours during the weekdays. The removal (in %) of marker micropollutants for individual samples and the average removal by E-peroxone is presented in Table 5 in comparison to average removal by ozonation. Conventional ozonation treated wastewater samples were collected during 18/11/2022 to 25/11/2022.

In general, the results followed the same pattern described in previous section 6.5 where all O_3-reactive micropollutants were removed either completely or showed very high removal. All moderately O_3-reactive micropollutants also showed very high average removal (>90%) while benzotriazole showed relatively low removal but still it was reaching to ~80%. In contrast, ozonation showed complete removal for all micropollutants except for benzotriazole where 11% decrease in removal was seen in comparison to E-peroxone.

The removal of O_3-resistant micropollutants by E-peroxone was significantly high as compared to ozonation. An increase of 43%, 13% and 22% in removal was achieved by E-peroxone as compared to ozonation for fluconazole, oxazepam and memantine, respectively. On the other hand, a small decrease of 7% was seen for irbesartan by E-peroxone than ozonation. This is mainly due to the effect of one sample collected on 25/11 where E-peroxone showed negative removal. This can be attributed to very low initial concentration of irbesartan on 25/11 i.e., 10 ng/L which is close to limit of quantification. After E-peroxone, the concentration remained in the same range and could possibly be below limit of quantification (LOQ). If excluding the sample with negative removal, the average removal of irbesartan by E-peroxone is 94% which is 10% higher that ozonation.

From full screening of 100 micropollutants, 32 pharmaceuticals were detected during the winter testing. The removal results of all 32 pharmaceuticals by E-peroxone process for the sample

collected on 14/11/2022 is presented in Table S1. As can be seen, very high removals for almost all detected micropollutants were achieved except for few compounds. The lowest removal was observed for bupropion (46%). Out of 32 detected, only 5 micropollutants were removed less than 80% while all other micropollutants were removed >80%. The average removal of all detected micropollutants was 91% which is well above the requirement of 80% reduction, recommended by authorities and is considered acceptable.

Table 5. Average removal of marker micropollutants in Strömsund wastewater effluent by E-peroxone process and ozonation during the winter testing, and the removal for individual samples by E-peroxone.

Compounds	*Average removal by ozonation (%)*	*Average removal by E-Peroxone (%)*	*Removal by E-peroxone (%) for individual samples during winter testing*					
			14/11	*16/11*	*18/11*	*21/11*	*23/11*	*25/11*
O_3-reactive group								
Paracetamol	100	100	100	ND	100	ND	ND	ND
Diclofenac	100	100	100	100	100	100	100	100
Trimethoprim	100	100	100	100	100	100	100	100
Carbamazepine	100	98	91	96	100	100	100	100
Moderate O_3-reactive group								
Metoprolol	100	93	100	100	100	100	86	72
Citalopram	100	98	89	100	100	100	100	100
Methyl-benzotriazole	100	94	79	100	100	100	82	100
Benzotriazole	68	79	61	100	77	74	83	77
O_3-resistant group								
Irbesartan	84	*77 (94)	100	100	100	84	87	-8
Memantine	73	95	84	100	100	86	100	100
Oxazepam	72	85	71	93	86	93	87	78
Fluconazole	55	98	100	100	100	100	100	89

ND: Not detected

* The value presented in parentheses is the average removal without the sample taken on 25/11.

6.6.2 Autumn Testing

The autumn testing was conducted during the period of 12/09/2022 to 23/09/2022 with the continuous run of the pilot system. The treated water samples were collected after almost 48 hours during the weekdays. The removal (in %) of marker micropollutnats for individual samples and the average removal by E-peroxone treatment is presented in Table 6.

The removal of O_3-reactive compounds showed similar results to winter testing and all micropollutants from this group were removed almost completely. Among moderately O_3-reactive micropollutants, citalopram and methyl-benzotraizole also showed very high average

removal (>90%). However, metoprolol and benzotriazole showed slightly low removal (78% and 69%, respectively) which is 15% and 10% decrease from the winter testing. The low removal of benzotriazole for this season can be related to very low removal on 12/09, thus resulting lower average removal.

The removal of O_3-resistant micropollutants by E-peroxone was slightly lower during autumn testing as compared to winter results. Irbesartan and oxazepam were removed by >80%. Memantine showed 70% removal (25% less removal from winter testing) while fluconazole showed 61% removal (37% less removal than winter). Average lower removal of fluconazole could be related to its low removal on 19/09 (7%) and 21/09 (30%) which resulted overall low removal. For the rest of the testing period, the removal of fluconazole was almost 80% or higher.

Table 6. Removal of marker micropollutants in Strömsund wastewater effluent by E-peroxone process during autumn testing for individual samples and their average removal.

Compounds	*Average removal by E-Peroxone (%)*	*Removal by E-peroxone (%) for individual samples during autumn testing*					
		12/09	*14/09*	*16/09*	*19/09*	*21/09*	*23/09*
O_3-reactive group							
Paracetamol	100	ND	100	100	ND	100	100
Diclofenac	100	100	100	100	99	100	98
Trimethoprim	97	93	100	90	100	100	100
Carbamazepine	98	97	98	98	100	99	98
Moderate O_3-reactive group							
Metoprolol	78	86	81	84	77	65	74
Citalopram	90	83	100	92	82	93	89
Methyl-benzotriazole	90	100	100	89	91	ND	68
Benzotriazole	69	27	79	83	70	72	81
O_3-resistant group							
Irbesartan	86	85	82	94	100	83	75
Memantine	70	52	75	85	68	65	73
Oxazepam	82	88	78	84	77	85	81
Fluconazole	61	86	86	77	7	30	81

ND: Not detected

The E-peroxone process and ozonation were compared again for removal of micropollutants for samples collected on 12/09/2022 during autumn testing, and the results are presented in Figure 7. The same effect of increased removal by E-peroxone was observed for O_3-resistant micropollutant. For instance, 24%, 19%, 12% and 48% increase in removal was seen for irbesartan, memantine, oxazepam and fluconazole, respectively by E-peroxone as compared to ozonation. The removal of benzotriazole was only 27% by E-peroxone as compared to 47% for ozonation. The lower removal was seen for benzotriazole for this particular sample, and it was always higher than 70% for rest of the samples.

From full screening of 100 micropollutants, 32 micropollutants were detected during the autumn testing and the removal results of individual micropollutants by E-peroxone process is presented in Table S1. As can be seen, very high removal for almost all detected micropollutants was achieved. The lowest removal was observed for benzotriazole i.e., 27%. Out of 32 detected, only 4 micropollutants were removed less than 80% while all other 28 micropollutants were removed >80%. The average removal of all detected micropollutants was 86% which is, slightly lower than winter results (91% removal), yet well above the recommendation of 80% reduction.

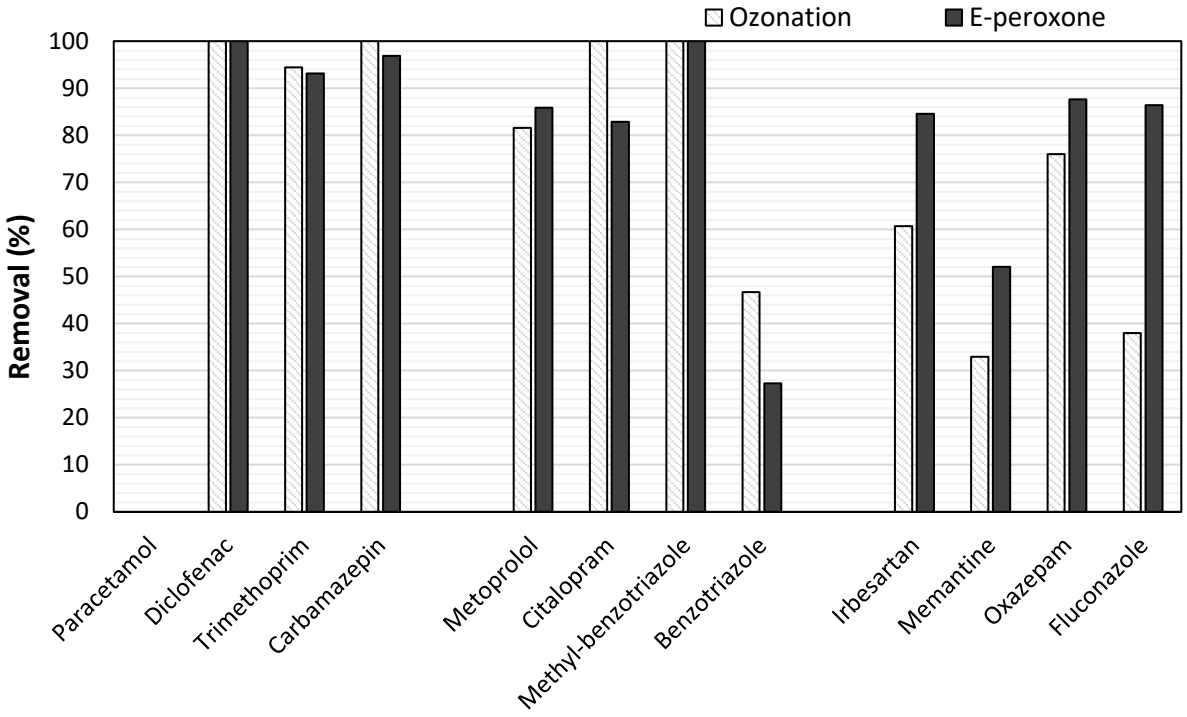

Figure 7. Removal of marker micropollutants by E-peroxone and ozonation for the samples collected on 12/09/2022.

6.6.3 Summer Testing

The Summer testing was conducted during the period of 18/07/2022 to 27/09/2022 and treated water samples were collected at different time points for two weeks. The removal (in %) of marker micropollutants for individual samples and the average removal by E-peroxone is presented in Table 7.

The removal of O_3-reactive compounds showed similar results to winter and autumn testing and all micropollutants from this group were removed almost completely except for trimethoprim (83% average removal). This is resulted from its low removal (35%) on 18/07. Among moderately O_3-reactive micropollutants, citalopram was completely removed while ≥82% removal was achieved for the other three micropollutants in this group.

The removal of O_3-resistant micropollutants by E-peroxone was higher during summer than autumn testing. For example, irbesartan and oxazepam were removed ≥90%. Memantine was removed to 74% and fluconazole removed 77%. No ozonation sample was collected for this season.

Table 6. Removal of marker micropollutants in Strömsund wastewater effluent by E-peroxone process during the summer testing for individual samples and their average removal.

Compounds	*Average removal by E-Peroxone (%)*	*Removal by E-peroxone (%) for individual samples during summer testing*				
		18/07	*20/07*	*21/07*	*26/07*	*27/07*
O_3-reactive group						
Paracetamol	100	ND	ND	ND	ND	100
Diclofenac	100	100	100	ND	100	100
Trimethoprim	83	35	100	100	81	100
Carbamazepine	99	100	100	96	98	100
Moderate O_3-reactive group						
Metoprolol	82	90	88	86	77	69
Citalopram	100	100	100	100	100	100
Methyl-benzotriazole	85	91	92	89	79	74
Benzotriazole	86	100	65	100	82	83
O_3-resistant group						
Irbesartan	90	89	100	58	100	100
Memantine	74	100	49	83	65	71
Oxazepam	98	100	100	90	100	100
Fluconazole	77	82	100	59	44	100

ND: Not detected

Out of 100 screened micropollutants, 38 micropollutants were detected during the summer testing and the removal results of these micropollutant by E-peroxone process is presented in Table S1. As can be seen, very high removal for almost all detected micropollutants were achieved. The lowest removal was observed for azelastine (19%) followed by trimethoprim (35%). Interestingly, both of these pharmaceuticals are O_3-reactive compounds. Except azelastine and trimethoprim, the removal of all other micropollutants were >80%. The average removal of all detected micropollutants was 94% which is, similar to previous results, well above the recommendation of 80% reduction.

6.6.4 Spring Testing

The installation and the optimization of the pilot system was done during the spring season so the testing period of E-peroxone with optimized conditions was kept short to one week i.e., 19/05/2022-24/05/2022. The treated water samples were collected at different time points during the testing period. The removal (in %) of marker micropollutants for individual samples and the average removal by E-peroxone is presented in Table 8.

Table 7. Removal of marker micropollutants in Strömsund wastewater effluent by E-peroxone process during the spring testing for individual samples and their average removal.

Compounds	*Average removal by E-Peroxone (%)*	*Removal by E-peroxone (%) for individual samples during summer testing*		
		19/05	*20/05*	*23/05*
O_3-reactive group				
Paracetamol	100	100	100	100
Diclofenac	100	100	100	100
Trimethoprim	99	100	100	96
Carbamazepine	100	100	100	100
Moderate O_3-reactive group				
Metoprolol	79	92	83	62
Citalopram	97	100	100	90
Methyl-benzotriazole	85	89	81	83
Benzotriazole	61	83	47	53
O_3-resistant group				
Irbesartan	73	100	55	64
Memantine	68	81	53	70
Oxazepam	60	77	36	65
Fluconazole	66	100	69	29

The removal of O_3-reactive compounds showed similar results to other three seasons and all micropollutants from this group were removed almost completely. Among moderately O_3-reactive micropollutants, citalopram, methyl-benzotriazole and metoprolol removed (avg.) 97%, 85% and 79%, respectively. Benzotriazole average removal was somewhat low, i.e., 61%. Similarly, the removal of O_3-resistant micropollutants by E-peroxone was lower (ranging from 60-73%) as compared to rest of the seasons. By looking at the removal results for each day, it can be observed that the removal of all these compounds on 19/05 is significantly higher than removals on 20/5 and 23/5. The reason is that no samples were collected on 20/5 and 23/5 for the concentrations of these micropollutants in the untreated water. To measure the removal (%) of a compound, concentration of the same compound in untreated water at the same is required when the treated water samples is collected. This implies that two samples are needed to collect at the same time to calculate removal where one is untreated, and the other samples is treated. Since the untreated samples were not collected, the removal of 20/5 and 23/5 samples was calculated using the untreated sample concentration from 19/5. As we have seen large variation in concentrations of micropollutant, this would have affected the removal results. In comparsion to these samples, the removal of micropollutants on 19/05 is significantly higher, which is similar to the results of all other seasons because the removal is based on micropollutants concentrations of untreated and treated sample at the same time. Thus, the most correct results for this season should be considered for the samples on 19/05. For the same sample, removal of micropollutants by E-peroxone is compared with ozonation and discussed earlier (see section 6.5 and Figure 6).

6.7 Bromate formation

Bromate is one of the by-products formed during ozonation which is formed by the reaction of bromides with O_3 and/or $^{\bullet}OH$ via series of reactions during ozonation (von Gunten and Hoigné, 1994). Bromate is a regulated carcinogenic compound that poses a significant threat to human health (Kurokawa et al., 1990). Several countries have regulated its concentration e.g., to 10 µg/L in drinking water (Von Gunten, 2003) and 50 µg/L according to the freshwater environmental quality standard (Soltermann, et al. 2016). One proposed method of minimizing bromate formation during ozonation is addition of ammonia (Hoffman and Andrew, 2001). Another way of inhibiting bromate formation during ozonation is addition of H_2O_2 which reduces HBrO/BrO^- (a key intermediate in bromate formation) to Br^- (Von Sonntag and Von Gunten, 2012).

Interestingly, no bromate was formed at any of the tested ozone doses i.e., 0.6, 0.9 and 1.1 gO_3/gDOC during ozonation treatment of Strömsund wastewater effluent, although the bromide level in Strömsund STP effluent was detected at 67µg/L which is sufficient for bromate formation (Jahan et al., 2021). In addition, previous studies have reported linear increase in bromate formation with specific ozone doses ≥ 0.4–0.6 mgO_3/mgDOC (Soltermann et al., 2016). The ozonation results for bromate formation in current project requires further investigation to evaluate the reason of no bromate formation. Similarly, no bromate was formed during E-peroxone treatment which was expected and reported previously (Li et al., 2015).

6.8 Toxicity removal

6.8.1 Acute toxicity in *Daphnia magna* (*in vivo*)

Toxicity in both untreated (effluent after conventional treatment steps, S102) was compared to unexposed controls and to effluents treated as by ozonation and E-peroxone. The tests were performed by BioImpakt AB, Örebro and is reported in Appendix 3. A summary of the results is presented below highlighting the most important findings.

Rather high acute toxicity in *Daphnia magna* was detected for the untreated effluent S102 with 55 % mortality in the highest exposure (100 % effluent). A large reduction in acute toxicity is seen for the treated effluents by ozonation (S103) and E-peroxone (S104) where acute toxicity is reduced to less than 30% for S103 and 25 % for S104. The results are shown in Figure 8. In Figure 8, corresponding acute test are also shown for Lycksele STP where the same tests were run and the outcome show a similar trend, which supports the conclusion on that treatment with advanced oxidation considerably lowers the toxic effects after treatment. In the Lycksele case, levels of pharmaceuticals were about 5 times higher which also suggest that pharmaceuticals could have contributed to the detected effects since the acute effects were higher for Lycksele samples as compared to Strömsund with 100 % mortality at 100 % effluent (L69, se figure 8). In the Lycksele case, reduction of acute toxicity was about 70 % for ozonation (L70) and about 85 % for e-peroxone treatment (L71, L80).

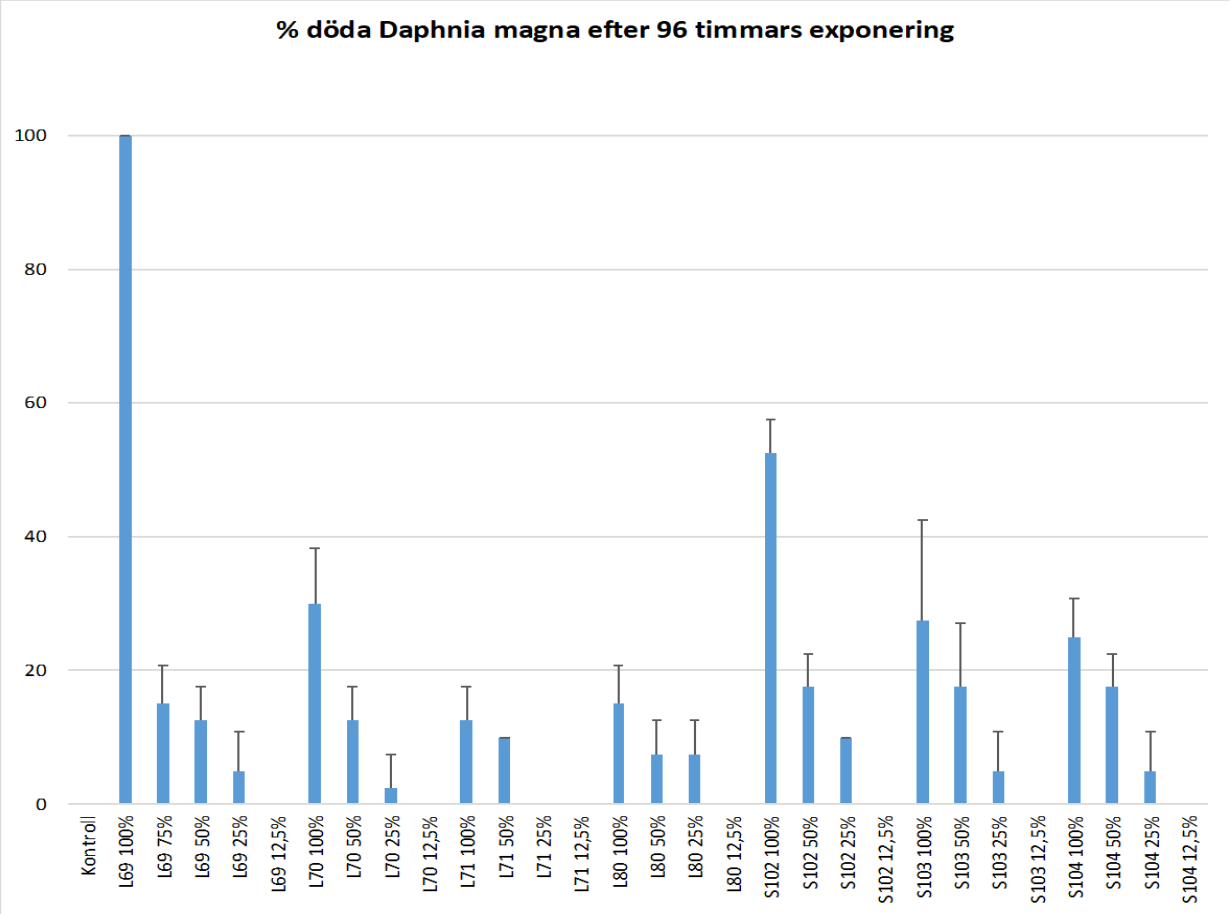

Figure 8. Acute toxicity and immobilisation in Daphnia magna in effluents from Strömsund and Lycksele STP.

6.8.2 Gene expression analysis in *Daphnia magna*

Analysis of gene expression using Genotox profile® based on qPCR analysis detected very little around any causal relationship to certain mechanisms för toxicity. The results are shown in Figure 9.

Effluent samples from Strömsund had in general low effects detected in the genotox profile tests in *Daphnia magna* and it was difficult to interpret statistically significant differences in gene expression between untreated (S102) to ozonation (S103) and E-peroxone treated (S104) samples.

A small difference is indicated in the expression of MTB and Cat which is down-regulated thus reflecting a reduced pressure from oxidative stress and metal exposure as compared to both control and untreated effluent. Since the oxidative stress is lowered in treated samples it can also be reasoned that no excess oxidants remain in the treated effluents, neither from ozonation or E-peroxone.

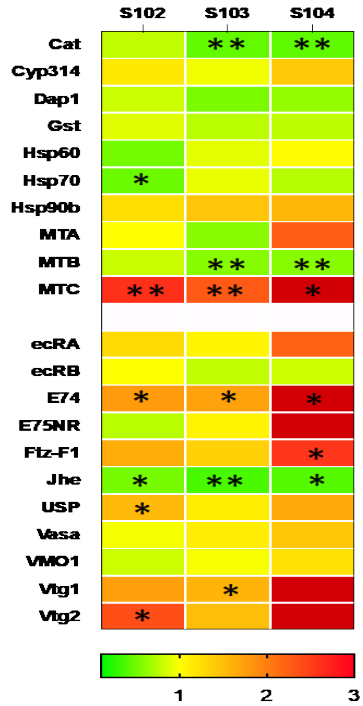

Figure 9. Genotox profile® analysis of gene expression in Daphnia magna post-exposure of water samples from Strömsund STP.

6.8.3 *In vitro* analysis in human cell lines

6.8.3.1 HepG2 cells

It was found that there is no difference in the gene expressions for marker genes reflecting disturbances in metabolism and phase transformation processes in lever cells. Only a weak statistical difference was seen for Sod3 possibly reflecting a small increase in oxidative stress and/or presence of organic metabolites in sample S104.

6.8.3.2 MCF7 cells

MCF7 cell tests aim to detect any estrogenic signalling, but also reflect exposure to organic xenobiotics (e.g., dioxins and PAHs). The results (shown in Figure 10) indicated that Cyp1A1 is affected in S102 (up-regulated) and S103 (down-regulated) for Strömsund samples. A small effect can thus be seen even though it is statistically weak relationship for this biomarker. Cyp1A1 in S104 was also slightly down-regulated, however, not statistically significant, see Figure 10.

For estrogenic signaling, a weak relationship is indicated for S103 (ozonation) who Erb gene is slightly up-regulated compared to the untreated water (S102). Since there are small differences between both treated samples (S103 and S104) and untreated it is difficult to interpret any strong trends and the presence of estrogenic compounds seem to be low in these waters. It should be noted that no estrogenic compound (e.g., 17 beta estradiol) was part of the screening performed for pharmaceuticals in the project.

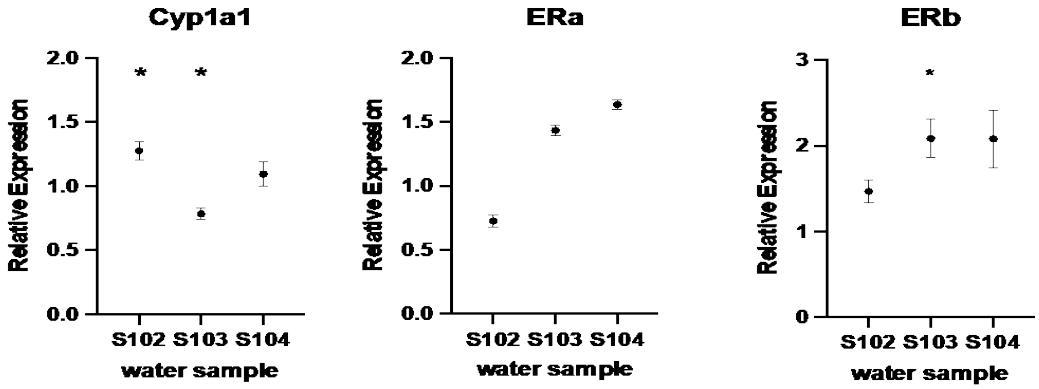

Figure 10. Analysis of gene expresssion in MCF7 cells after exposure to untreated and treated waters from Strömsund STP.

6.8.3.3 Analysis of immune response in THP1 cells

Exposure of THP1 cells for different effluents without pre-induction using LPS show that IL-6 base level is slightly elevated av e-peroxone treatment (S104). After induction of the immune response with LPS no change can be seen compared to the control (LPS), see Figure 11.

The TNF-a expression levels were affected in all water samples from Strömsund. Here, a general induction of TNF-a could be seen in both ozonation- and e-peroxone treated samples. Following LPS induction, a strongly increased signal was seen after e-peroxone treatment (S104), see Figure 11.

The THP1 cell tests indicate presence of immune disturbing substances in the effluents and besides TNF alfa induced and increased activation in S104 small differences are seen between treated and untreated, thus advanced oxidation seem to not cause any signicant reduction in this signalling. It could therefore be other constituents in the effluents causing the detected effects on immune response. Since the reduction in the levels of pharmaceuticals is largely reduced by advanced oxidation, these immune effects are more likely to cause by other agents in the waters.

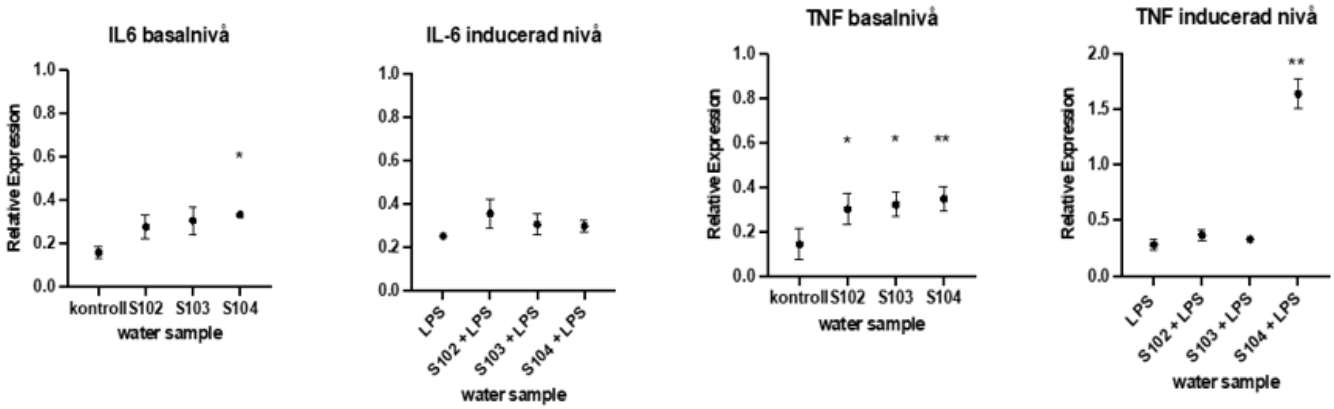

Figure 11. Analysis of protein levels of immune response for biomarkers IL6 and TNF-a in THP1 cells post-exposure to untreated and treated waters from Strömsund STP. Effects on both base levels and LPS-induced levels were tested.

7 OPERATING COST AS ELECTRICAL ENERGY CONSUMPTION

The operating cost is one of the key considerations for full-scale application of advanced oxidation processes. For conventional ozonation system, the operating cost of a system is dominated by the energy consumption which is very much dependent on the design of the system. Similarly for in situ electrochemical generation of H_2O_2 during E-peroxone process, the energy consumption is dependent upon the efficiency of the electrodes that is measured as current efficiency of H_2O_2. Due to varying current energy prices in different parts of Sweden, instead of converting energy into currency, the operating costs are discussed by energy consumption (in kWh).

The energy consumption for current pilot was calculated using the same method reported by (Nilsson, 2015). The energy consumption for conventional ozonation with current pilot for treating one cubic meter of water was estimated to be ~0.251 kWh m^{-3} $year^{-1}$ which is slightely higher than filip nilsson 2015 i.e., 0.212 kWh m^{-3} $year^{-1}$. This is because few components in current pilot have over capacity thus were consuming more energy than required.

On the other hand, the energy consumption requirement for in situ electrochemical generation of hydrogen peroxide, at $[O_3]$:$[H_2O_2]$: 0.5, is estimated to be ~0.092 kWh m^{-3} $year^{-1}$. So, the total energy required for E-peroxone process is estimated to be 0.343 kWh m^{-3} $year^{-1}$. It is worth mentioning that recent improvements in the electrochemcal cell have resulted in doubled the production of H_2O_2 with almost same energy consumption. As a result, the energy consumption for in situ electrochemical generation of hydrogen peroxide at the same ozone and H_2O_2 ratio would already decrease to ~0.046 kWh m^{-3} $year^{-1}$. Further optimization of $[O_3]$ and $[H_2O_2]$ ratio can also reduce the dose of hydrogen peroxide, hence the energy requirement for E-peroxone process. It can be concluded that the electrical energy consumption by E-peroxone will not vary significantly from ozonation.

8 DISCUSSION AND CONLUSIONS

Sewage treatment plants with conventional treatment processes are acting as hot spots for the spread of micropollutants into the environment. The reason is that conventional STPs are not designed and equipped for removal of micropollutants. Strömsund STP is one example which is releasing ~40 kg of pharmaceuticals every year (based on screening of only 98 pharmaceuticals and 2 benzotriazole in this project) into the environment, in this case Lake Ströms Vattudal. Considering the 1200 pharmaceuticals sold in Swedish market and the same detection ratio, the actual pharmaceuticals mass released by Strömsund STP in Lake Ströms Vattudal is expected to be much higher. Furthermore, pharmaceuticals represent only one class of micropollutants if consider other classes of micropollutants such as biocides, personal care products, poly aromatic hydrocarbons, perfluoroalkyl substances etc. and their release from STPs, the impact must be substantially larger for the environment. Micropollutants adverse effects, particularly pharmaceuticals, both for humans and the environment are generally recognized, and upgrading STPs with facilities enabling advanced tertiary treatment, including removal of micropollutants, is essential.

A recently developed E-peroxone process was tested on industrial scale for removal of micropollutants in Strömsund STP effluent and compared it with already established conventional ozonation process. Ozonation was able to remove selective micropollutants due to selctive nature of ozone. Many persistent micropollutants showed low removal by ozonation as their removal is influend by the structural features that does not react with ozone. However, relatively slightly higher removal of micropollutants by ozonation in this project as compared to

other studies, especially for those which have low ozone reactvity, can be connected to two injection strategy of ozone, appropriate ozone dose and longer treatment. On the other hand, E-peroxone process was able to achieve significantly higher removal for all micropollutants including O_3-resistant micropollutants as compared to ozonation. Likewise, moderately O_3-reactive compounds were also effeciently removed by E-peroxone. The results from the continuous test run showed that the process worked robustly under different seasons and varying testing conditions as only small seasonal variations in results were detected. Overall, E-peroxone process was able to remove all pharmaceuticals (detected in Strömsund STP effluent) in the range of 86-94% for all seasons. Many other projects have been studying micropollutants that are mostly ozone reactive. However, a large number of micropollutants are expected to be persistent to conventional ozonation. For instance, we developed a quantitative structure activity relationship model in a scientific article to predict the ozone reactivity of 491 pharmaceuticals present in Swedish market (Mustafa et al., 2021). The model suggested approx. >150 pharmaceuticals to be persistent to ozonation. Thus, the removal of these persistent pharmaceuticals are expected to be improved by E-peroxone process as suggested by the results in this work and other scientific studies (Cruz-Alcalde et al., 2020; Guo et al., 2015; Mustafa et al., 2021; Wang et al., 2019).

Although it is difficult to show or prove the actual level of oxidation and degradation of a certain parent compound, a stronger oxidation process presumably should move closer to complete mineralization of the parent compound if compared to a weaker oxidation method. Therefore, it can be reasoned that E-peroxone has an advantage over ozonation in that aspect. Further, when not reaching full mineralization, smaller metabolites and fragment are likely to be more prone to further abiotic and biotic degradation processes after entering the aquatic environment. Additionally, it would also lead to decreased presence of pharmaceutical residues that are in any bioactive form. Overall, it would therefore be advantageous to choose a stronger advanced oxidation method e.g. E-peroxone in that perspective.

A comprehensive risk assessment, and the implications for the recipient after introduction of tertiary treatment with E-peroxone was not part of this pre-study and its project plan. However, a few remarks will be made below on the complexity of performing such assessment for both environment and human health regarding emissions of pharmaceuticals.

The receiving water system Ströms Vattudal and Faxälven has an average flow of approx. 151 m^3/s accordning to SMHI (https://www.smhi.se/data/hydrologi/vattenwebb). The flow at Strömsund STP is on average ~1500 m^3/24 h (Strömsunds kommun, Annual report 2019, Strömsunds STP) which corresponds to approx. 0,018 m^3/s. This would imply an average dilution factor of sewage wastewater effluent of almost 8400. However, in the vicinity of emissions points and close to a sewage treatment, much lower dilution occurs. Dilution is very dependent on local conditions also as depth, flow directions and overall flow dynamics. Hence, it is very difficult to estimate actual dilution and there will be seasonal variations due to flow variations.

Consequently, there will always be uncertainties tied to such estimations on which pharmaceuticals will enter an aquatic system at a certain time and how the fate of pharmaceutical residues will turn out. Out of the many existing and sold pharmaceuticals, only a few can be analysed in regular laboratories. Besides pharmaceuticals a large number of other contaminants circulates via the sewage. In this study, out of 98 screened pharmaceuticals, 41 were detected in Strömsund STP effluent. Further to get an idea of actual situation in term of diversity of micropollutants occurrence at STP, two biocides were also analysed and interestingly, both of them were dectected as the most common compounds during all season. Compared to derived critical environmental concentrations (CECs) of pharmaceuticals (Fick et

al, 2010), only a handful of detected pharmaceuticals are above these values in untreated effluent, thus indicating a potential risk. However, such CEC values are based on individual substances physico-chemical properties and known ecotoxicological no effects levels, PNECs (predicted no effect concentrations). For many pharmaceutical substances, PNECs are based on acute ecotoxicity measures, hence the chronic effect levels remain unknown. When prediciting or setting a PNEC as basis for environmental quality criteria, assessment factors are usually applied to cope with uncertainties regarding chronic effects.

CEC-values are formed on a basis of known human therapeutic plasma levels and they are primarily based on effects from acute toxicity data and exposure from short-term treatment regimes mainly targeting fish species. This is important from several aspects. Firstly, it may not cover the difference in intraspecies sensitivity towards different substances e.g. human, to fish to invertebrates to algae etc.. Secondly, it may not at all cover long term bioaccumulation effects potentially causing much higher tissue levels compared to concentrations found in the aquatic media. Thirdly, obvious uncertainties remain on the potential mixed exposure effects that may occur when different xenobiotics act via the same mechanisms and toxicological target. Further, risk assessment will be immensely complicated as different recipients and surface waters will vary greatly both in their ecological values and physico-chemical conditions which largely affect intrinsic bioavailability and sensitivity towards any bioactive agents.

Recent publications from several groups as acknowledged in the background section have shown some worrying trend where trophic transfer of biomagnifying substances have been demonstrated and where increased tissue concentrations in aquatic species have been found. This could imply a possible trophic transfer not only to aquatic top predators, but also to the terrestrial food chain, thus enabling different exposure routes for humans. This have been shown at the same time as concentrations in the water phase have been found in low to non-detectable levels in the studied recipients. The recent study by Richmond et al (2018) were conducted in Australian waters nearby Melbourne, and as indicated by Wilkinson et al (2022), have concentrations in the lower range of pharmaceutical loadings in surface waters in a global comparison. However, this may still have potential risks related to pharmaceuticals presence at these levels.

In current project, different bioanalytical tools, both *in vivo* and *in vitro*, were used to reflect the effects and benefit from tertiary treatment as by E-peroxone compared to the more common ozonation process. Ozonation has been shown to reduce toxicity largely in many different studies for sewage effluents. The most extensive review and compilation can be found in Völker et al (2019) and presents high removal of toxicity measured by many biological endpoints as by ozonation compared to only conventional treatment processes at STPs. Examples in are given for removal of effects covering estrogenicity, androgenicity, anti-estrogenicity, anti-androgenicity , glucocorticoid and progestogenic activity , aryl hydrocarbon receptor activity, pparg activity, adaptive (oxidative) stress response, acetylcholinesterase inhibition, combined algae assay, bioluminescence inhibition, retinoid-like activity, steroidgenesis, CAR and PXR receptor activity, ROS formation, UmuC assay, SOS chromotest, Ames assay, phytotoxicity (in vivo), invertebrate toxicity (in vivo) and fish toxicity (in vivo). The Völker study was based on reviewing 2464 publications and extraction of data from 46 relevant studies and the studies from 22 pilot or full-scale STPs.

In this project, a well-established and sensitive ecotoxicological model was chosen by studying the invertebrate Daphnia magna both for acute effects and analysis of reproductive and toxicological gene expression. In addition, effect-based methodology as proposed by Brack et al (2019) were applied to explore relative differences between untreated and treated samples. Some human cell line In vitro assays addressing different modes of action on prioritized mechanisms for reflecting xenobiotic exposure were used in an attempt to cover for some of the most relevant effects that potentially can be found in sewage effluent. In vitro tests and gene

expressions analysis overall gave few conclusive answers on differences between different treatment schedules. However, significant differences were shown from the acute tests for *Daphnia magna* where obvious benefits from E-peroxone were demonstrated. Further, the overall reduction in toxicity was clearly also higher for E-peroxone. The above-described battery of bioanalytical tools should not be considered as comprehensive, however, the battery covers for many of type of effects that is recommended to be studied in this kind of evaluation and is more than many other studies previously have included. But for a better understanding of the fate of pharmaceuticals in this particular lake system, more extensive analysis must be performed. This is to better understand both the variations in pharmaceuticals concentrations during different seasons as well as levels of target contaminants in aquatic biota.

Regarding the scalability of peroxone process in which H_2O_2 is provided externally, peroxone process has already been tested. However, continuous supply of H_2O_2 especially to remote areas, handling and storage of H_2O_2 are few big challenges which makes peroxone less popular due to sustainability issues. In comparison, E-peroxone process provides a sustainable approach of generating H_2O_2 electrochemically on site at the expense of air and electricity. Interestingly, the cost of in situ electrochemical production of H_2O_2 has been reported similar to externally supply. Li et al (2021) estimated the overall cost of electrochemical production of H_2O_2 to be about 0.88 $/kg H2O2 which is economically competitive to the externally supply, i.e., 0.7-1.2 $/kg H2O2 (Ciriminna et al., 2016).

Current pilot system was treating ~215 m^3/day of Strömsund and the scalability of E-peroxone process does not seem to be a problem for majority of the STPs in Sweden. For instance, Sweden has in total 1700 STPs of different sizes. Among them, approximately 1550 STPs are categorized in the range of up to 10 000 pe which is almost 91% of the total STPs in Sweden (Naturvårdsverket, 2017). With current efficiency of the electrodes to electrochemically generate H_2O_2, the E-peroxone process can treat water flows of STPs upto 10 000 pe with the same water quality. Strömsund STP, dimensioned for 7000 pe, also fall in the same category. For large STPs with very high flows, parallel installations serving the main flow can be considered. In addition, the replacement cost of electrodes after every year should take into consideration when evaluating the feasibility E-peroxone process for full scale applications.

Testing of E-peroxone process for Strömsund and Lycksele STPs with varying water characteristics and different primary and secondary treatment processes indicates that it can cope under different conditions and less sensitive to changes in the incoming water. However as indicated by the results from these projects, E-peroxone process requires optimization for seasonal variations. Due to the possibility of retrofitting ozonation to E-peroxone process, existing STPs with ozonation can also be adapted to E-peroxone process.

In conclusion, when planning for tertiary treatment installations targeting pharmaceuticals, weighing risk and making cost-benefit analysis of such project, one must therefore carefully consider all above listed uncertainties. Future investments should take long-term safety of human health and the environment into account and from current knowledge preferably use precautionary principles in lack of facts and sound data.

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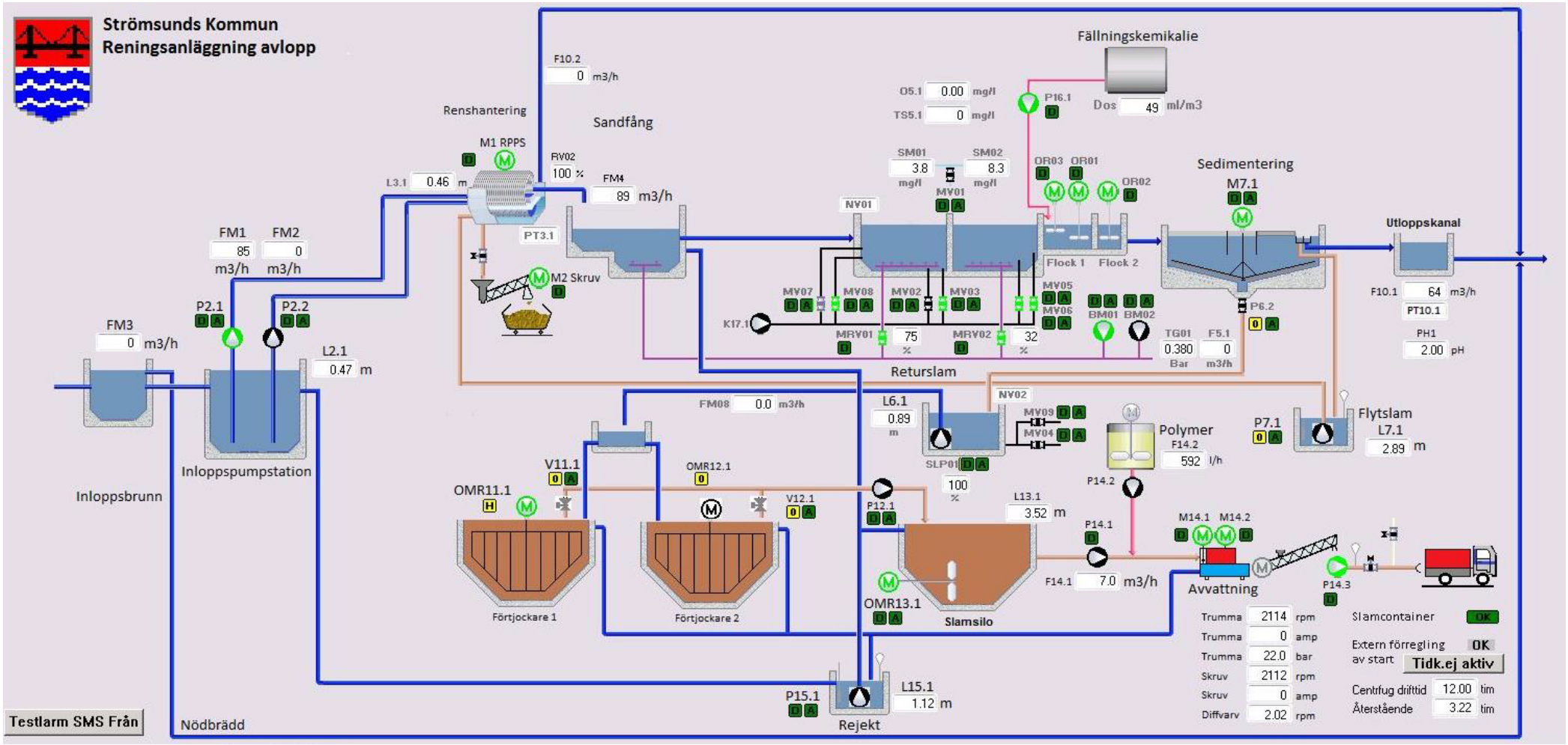
Appendix 1

Table S1: Removal (%) of all detected micropollutants in Strömsund wastewater effluent by E-peroxone during the three seasons and the average removal in each season.

	Winter Testing	**Autumn Testing**	**Summer Testing**
Pharmaceutical	*Removal (%) on 14/11/2022*	*Removal (%) on 12/09/2022*	*Removal (%) on 18/07/2022*
Alfuzosin	100	100	100
Amitriptyline	100	100	100
Atenolol	100	93	100
Atorvastatin	100	100	100
Azelastine	ND	ND	19
Azithromycin	ND	ND	100
Benzotriazole	61	27	100
Bisoprolol	89	86	97
Buprenorphine	ND	ND	100
Bupropion	46	43	ND
Caffeine	81	81	94
Carbamazepine	91	97	100
Cetirizine	100	96	100
Citalopram	89	83	100
Clarithromycin	ND	ND	100
Clindamycin	100	81	100
Codeine	100	100	94
Desloratadine	ND	70	100
Diclofenac	100	100	100
Diltiazem	100	100	100
Dipyridamole	ND	ND	100
Fexofenadine	86	91	93
Flecainide	65	84	89
Fluconazole	100	86	82
Fluoxetine	ND	ND	100
Irbesartan	100	85	89
Losartan	100	99	92
Memantine	84	52	100
Methyl-Benzotriazole	79	100	91
Metoprolol	100	86	90
Metronidazole	100	ND	ND
Mirtazapine	100	100	100
Oxazepam	71	88	100
Paracetamol	100	ND	ND
Propranolol	ND	ND	100
Rosuvastatin	100	91	100

Sertraline	100	81	ND
Sotalol	ND	ND	100
Sulfamethoxazole	100	100	ND
Tramadol	ND	86	91
Trimethoprim	100	93	35
Venlafaxine	82	90	100
Zolpidem	ND	ND	100
Total number of micropollutants detected	***32***	***32***	***38***
Average removal (%)	***91***	***86***	***94***

ND: Not detected


Strömsunds Kommun
Reningsanläggning avlopp
Fällningskemikalie
F10.2
0 m3/h
O5.1 0.00 mg/l
TS5.1 0 mg/l
P16.1
Dos 49 ml/m3
Renshantering
Sandfång
M1 RPPS
RV02
100 %
L3.1 0.46 m
FM4
89 m3/h
SM01 3.8 mg/l
SM02 8.3 mg/l
OR03 OR01 OR02
MV01
NV01
Sedimentering
M7.1
Utloppskanal
FM1 85 m3/h
FM2 0 m3/h
PT3.1
M2 Skruv
Flock 1
Flock 2
P6.2
F10.1 64 m3/h
PT10.1
PH1 2.00 pH
FM3 0 m3/h
P2.1
P2.2
L2.1 0.47 m
MV07 MV08 MV02 MV03 MV05 MV06
K17.1
MRV01 75 %
MRV02 32 %
BM01 BM02
TG01 0.380 Bar
F5.1 0 m3/h
Returslam
FM08 0.0 m3/h
L6.1 0.89 m
NV02
MV09 MV04
SLP01
100 %
Polymer
F14.2 592 l/h
P14.2
P7.1
Flytslam
L7.1 2.89 m
Inloppsbrunn
Inloppspumpstation
V11.1
OMR12.1
OMR11.1
V12.1
P12.1
L13.1 3.52 m
P14.1
M14.1 M14.2
F14.1 7.0 m3/h
Avvattning
P14.3
OMR13.1
Förtjockare 1
Förtjockare 2
Slamsilo
Trumma 2114 rpm
Trumma 0 amp
Trumma 22.0 bar
Skruv 2112 rpm
Skruv 0 amp
Diffvarv 2.02 rpm
Slamcontainer OK
Extern förregling av start OK
Tidk.ej aktiv
Centrfug drifttid 12.00 tim
Återstående 3.22 tim
P15.1
L15.1 1.12 m
Rejekt
Testlarm SMS Från
Nödbrädd

BioImpakt AB

Biologiska analyser av vatten renat med ozonering och e-peroxone från Strömsund avloppsreningsverk

RAPPORT BioImpakt AB Örebro 20221229

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BioImpakt AB

BESKRIVNING AV UPPDRAGET
BioImpakt AB har på uppdrag av Envix Nord AB utfört ekotoxikologiska tester på renat avloppsvatten från Strömsunds avloppsreningsverk. Följande delmoment ingår i utredningen:

- Akut toxicitet på *Daphnia magna*
- Analys av toxicitetsmekanismer hos *Daphnia magn*a med Genotox profile®
- Analys av toxicitetsmekanismer hos HepG2 celler med Genotox profile®
- Analys av reglering av ERα, ERβ och Cyp1a1 hos MCF-7 celler
- Analys av immunsvar med IL-6 och TNF-α med THP-1 celler
- Sammanställning och redovisning av resultat

UTFÖRDA ANALYSER

Biologiska analyser
Analys av biologiska effekter görs med utvalda modellsystem enligt förordning (EG) nr 440/2008 eller enligt andra internationellt erkända testmetoder och riktlinjer samt, i fråga om försök på djur eller människor, med hänsyn till artikel 7 i förordning (EG) nr 1272/2008.

Analyspaket 1:
Analys med *Daphnia magna* är baserad på OECD test 202, *Daphnia sp. Acute Immobilisation Test*. Resultaten redovisas som koncentration av prov som ger 50% inhibering av mobilitet (IC_{50}).

Analyspaket 2:
Analys av toxicitetsmekanismer hos *Daphnia magna.* För att identifiera kopplingar mellan exponering och effekt i organismen tillämpas analys av genuttryck.
För analys av genuttryck (*Genotox profile*®) exponeras *Daphnia magna* i 24 timmar. qPCR analyserna utförs enligt CEN/TS 17883:2022 samt ISO 20359. RNA extraheras och omvandlas till cDNA för efterföljande mätning av genuttryck som utförs för ett urval av gener. I denna utredning har fokus legat på gener som beskriver processer kopplade till reproduktion och generell toxicitet hos *Daphnia magna*.

Analyspaket 3:
Analys av toxicitetsmekanismer hos humana HepG2 celler. För att identifiera kopplingar mellan exponering och effekt i organismen tillämpas analys av genuttryck. För analys av genuttryck (*Genotox profile*®) exponeras HepG2 celler i 24 timmar. RNA extraheras och omvandlas till cDNA för efterföljande mätning av genuttryck som utförs för ett urval av gener. I denna utredning har fokus legat på gener som beskriver processer kopplade till generell toxicitet hos humana HepG2 celler.

HepG2 är en odödlig cellinje som härleddes 1975 från levervävnaden hos en 15-årig kaukasisk man från Argentina med ett väldifferentierat hepatocellulärt karcinom. HepG2-celler är ett lämpligt in vitro-modellsystem för studier av polariserade humana hepatocyter. Hep G2-celler är ett lämpligt *in vitro*-modellsystem för studier av polariserade humana hepatocyter.

Analyspaket 4:
Analys av störningar på östrogen signalering hos humana MCF7 celler. För att identifiera kopplingar mellan exponering och effekt i organismen tillämpas analys av genuttryck. För analys av genuttryck exponeras MCF7 celler i 24 timmar. RNA extraheras och omvandlas till cDNA för efterföljande mätning av genuttryck som utförs för ett urval av gener. I denna utredning har fokus legat på gener som beskriver processer kopplade till östrogen reglering hos humana HMCF7 celler.

MCF-7 är en bröstcancercellinje som isolerades 1970 från en 69-årig vit kvinna. MCF-7 är akronymen för Michigan Cancer Foundation-7, och syftar på institutet i Detroit där cellinjen etablerades 1973 av Herbert Soule och medarbetare. Cellinjen ger ett proliferativt svar på östrogener och östrogenagonister.

Analyspaket 5:
Analys av immunsvar hos humana THP1 celler. För att identifiera kopplingar mellan exponering och effekt i organismen tillämpas analys av proteinnivåer med ELISA. I denna utredning har fokus legat på gener som beskriver processer kopplade till reglering av IL-6 och TNF-α hos humana THP1 celler.

THP-1 är en human monocytisk cellinje som härrör från en patient med akut monocytisk leukemi, som har använts i stor utsträckning för att studera monocyt-/makrofagerfunktioner, mekanismer, signalvägar och transport av näringsämnen och läkemedel. THP-1-celler kan differentieras till makrofagliknande celler som liknar egenskaper hos mogna makrofager genom aktivering av proteinkinas C (PKC) med forbol-12-myristat-13-acetat (PMA), vilket slutligen resulterar i celler med ökad vidhäftning. Denna cellinje har blivit en vanlig modell för att uppskatta modulering av monocyt- och makrofagaktiviteter.

Statistik analys
Utvärdering av svaren utfördes med ANOVA följt av Dunnett's post-test för jämförelse mellan prov och referens. Statistisk signifikans indikeras för gener där medelvärdet ändras med mer än 50 % samt där signifikansen överstiger 95% (* $p<0,05$) eller mer än 10 % där signifikansen överstiger 99% (** $p<0,01$).

Kemiska bakgrundsdata erhållna från Envix Nord AB
Uppmätta värden av utvalda ämnen i ursprungsvatten/efter ozonering / efter e-peroxone behandling återges i tabell nedan.

Tabell 1. Koncentrationer av läkemedelsubstanser I testade vattenprover.

Substans	Site	Strömsund ARV		
	Prov ID	S102	S103	S104
	LOQ	Obehandlat vatten	Ozonering	E-peroxone
	ng/L	ng/L	ng/L	ng/L
Alfuzosin	4	164	11	<LOQ
Atorvastatin	10	250	<LOQ	<LOQ
Bisoprolol	3	510	72	72
Clarithromycine	3			
Clindamycine	3	44	<LOQ	8
Codeine	15	1238	<LOQ	<LOQ
Diclofenac	10	1975	<LOQ	<LOQ
Diltiazem	1,5	34	<LOQ	<LOQ
Fexofenadine	10	1110	47	98
Flecainide	1,5	134	20	22
Paracetamol	30			
Rosuvastatin	20	823	142	78
Sulfamethoxazol	15	186	<LOQ	<LOQ
Tramadol	15	991	18	141
Trimethoprim	3	260	14	18
Venlafaxine	20	1300	85	136
Propranolol	20			
Amytriptyline	10	62	<LOQ	<LOQ
Atenolol	15	889	149	66
Bupropion	3	104	80	59
Carbamazepin	7,5	987	<LOQ	31
Citalopram	15	281	<LOQ	48
Metoprolol	15	7102	1310	1002
Mirtazapine	15	265	<LOQ	<LOQ
Caffeine	20	49154	9459	9290
Ceterizine	15	424	<LOQ	18
Losartan	10	2671	<LOQ	31
BZ	50	1443	769	1049
MBZ	50	192	<LOQ	<LOQ
Desloratidin		93	<LOQ	28
Fluconazole	7,5	86	53	12
Irbesartan	3	70	28	11
Memantine	3	136	91	65
Oxazepam	10	535	128	66
Sertraline	10	126	<LOQ	25

Bakgrundsinformation till genotox profile® analysmetoden
För utvärdering av effekter orsakade av exponeringen har ett urval av gener, centrala för olika fysiologiska processer, analyserats med avseende på upp eller nedreglering. Genregleringen är organismens svar på behandling (exponering för testmediet), där den eftersträvar att bevara homeostas (stabilt fysiologiskt tillstånd i balans). Därför är en uppreglering eller nedreglering inte i första hand ett mått på toxicitet utan en identifiering av om en behandling resulterar i ett svar hos organismen. Mätning av upp och nedreglering av gener som reflektion och påverkan på centrala funktioner för organismens ”hälsostatus” är känsligare mått för påverkan än ett konventionellt test där t.ex. immobilitet, mortalitet eller tillväxthämning mäts. Därför kan påverkan och störning på genreglering erhållas utan att det nödvändigtvis påvisas akuttoxiska effekter i mer konventionella ekotoxikologiska tester. Genanalysens styrka jämfört med traditionella fysiologiska analyser är man får ett svar både vad gäller toxicitet, samt typ av mekanism som störs. Med kunskap om de olika genernas reglering kan man utreda orsaks-verkan samband. Kombinationen av de beskrivna testbatterierna ger en god bild av hur farligt/giftigt mediet är som genomgår testning både avseende om det är sannolikt att akuttoxiska effekter ska uppstå vid exponering, och om det kan förväntas andra biologiska effekter som uppträder innan de akuta.

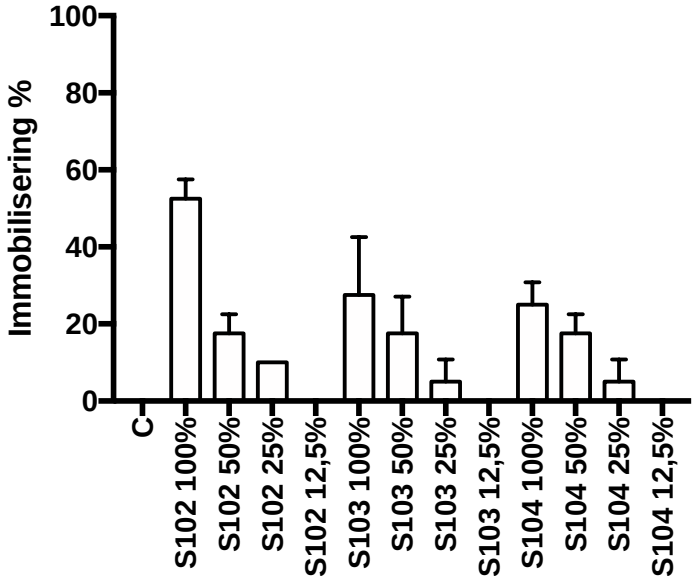
Vattenloppa är vanligtvis bland de känsligaste organismerna i en s.k. artkänslighetsfördelning där olika arters effektmått för ett visst medium eller agens jämförs. Uppnås ingen effekt på vattenloppa är det mindre sannolikt att samma testkoncentration ska ge upphov till negativa effekter på andra organismer.

RESULTAT
Resultat från utförda biologiska tester för obehandlat vatten, som enbart genomgått rening via konventionella reningssteg i avloppsreningsverket, jämförs med vatten behandlat med antingen ozonering eller e-peroxone.

Biologiska analyser
För att utreda vid vilken spädning som lakvattnet upphör att ge biologiska effekter har tre olika analyser utförts. Först har akuttoxicitet analyserats efter 96 timmar för att erhålla IC_{50} och IC_5 värden. Baserat på resultaten från dessa analyser har genotox profile® analyser utförts. Samtliga analyser har utförts på vattenloppa (*Daphnia magna*).

Daphnia magna
Daphnia magna är en sötvattenslevande hinnkräfta (vattenloppa) som filtrerar sin föda och därför kan påverkas av både lösta och partikelbundna ämnen. Den utgör en väldigt känslig art och är en bra indikatororganism för vatten.

Akuttoxicitetstest
För att utvärdera om lakvattnen med spädningar hade akuttoxiska effekter på *Daphnia magna* utfördes OECD test 202. En spädningsserie utgående från lakvatten.



Figur 1. Akut toxicitet uppmättes genom att analysera immobilisering hos Daphnia magna. IC_{50} värdet beräknades med hjälp av online verktyget AAT Bioquest.

Efter 96h uppmättes akuttoxiciteten för koncentrationer från 100% provvatten till 0% provvatten. För vatten från Strömsund (S) uppmättes ett IC_{50} värde på just under 100% för S102 d.v.s. i ett icke utspätt vatten som endast genomgått konventionell avloppsrening uppnås drygt 50 % mortalitet. För S103 och S104 kunde inga LC_{50} värden uppmätas då effekten var låg.

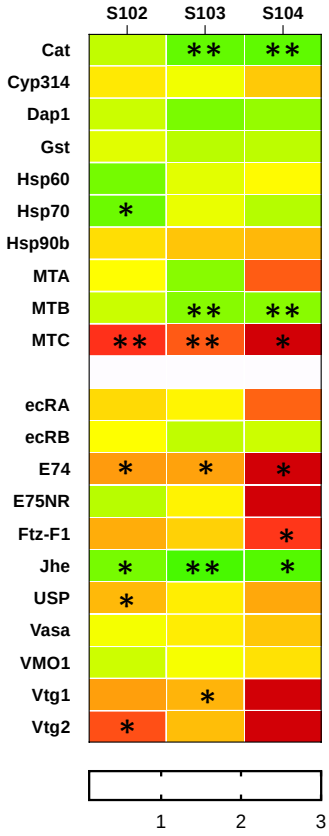
Utifrån akuttoxiciteten kunde LC_5 värden uppskattas. För vatten från Strömsund uppskattades LC_5 till ca 17% för S102, ca 25% för S103 och ca 25% för S104.

Från dessa resultat användes 100% vatten för samtliga genotox profile® analyser på *Daphnia magna*.

Genotox profile® analys med Daphnia magna

Analys av genuttryck med genotox profile® metoden baseras på qPCR analys av ett antal gener för ekotoxicitet. För ekotoxicitet inkluderas gener som svarar på generell toxicitet, metaller och fria radikaler, metabolism och celldöd. I en lista och förklaring till ingående geners funktion återfinns som appendix sist i rapporten.

Resultaten från genanalyserna presenteras i en ”heat map” där färgen på rutan visar om genen har blivit nedreglerad (grönt), uppreglerad (rött) eller är opåverkad (gult). Förutom att ange förändringen så är även statistisk analys inkluderad i figuren. En stjärna (*) avser $p<0,05$ och två stjärnor (**) avser $p<0,01$. Genom en kombination av antal påverkade gener, storleken på förändringen och den statiska signifikansen kan man avgöra hur stor påverkan som exponeringen har på genuttrycket hos *Daphnia magn*a jämfört med en icke exponerad kontroll.



Figur 2. Genotox profile® analys av genuttryck hos Daphnia magna efter exponering för vatten från Strömsunds reningsverk

Analys av lakvattnets effekt på genuttryck jämfört med ”Daphnia standard water”, kontrollvatten visar för proverna från Strömsund att effekterna på *Daphnia magna avseende* genreglering *är* förhållandevis små. Vatten från Strömsund hade mindre effekter utav obehandlat vatten gällande generell stressrespons sett till HSP70 genen, men även ökning hos behandlade prover avseende t.ex. metallothionein (MTB) och oxidativ stress (Cat) vilka uppreglerades efter behandling med både ozonering (S103) och e-peroxone (S104). Svag ökning kan även ses i obehandlat prov för uttrycket av generna USP och JHE, i bägge fallen kopplade till mekanismer viktiga för reproduktionen, men signalen är svag jämfört med de behandlade prover (låg signifikans). Tydligast effekt är således en ökning i genuttrycket för gener kopplade till stressrespons från metaller (Cat, MTB) vilket kan ha logik i att oxidativ behandling kan frisätta metaller som är bundna i organiskt material för obehandlat vatten. Sekundärt kan metaller medföra oxidativ stress som utrycks genom uppregleringen av Cat.

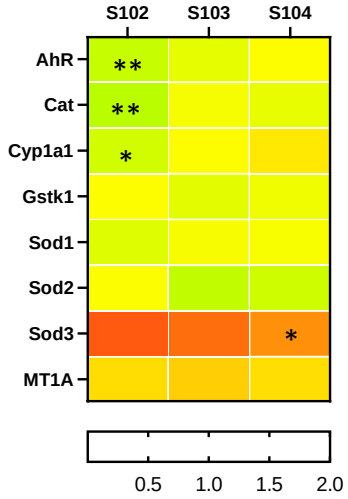
In vitro analyser med humana cellinjer

Åtta olika gener analyserades hos HepG2 celler. Dessa var cytokrom P450 1A1 (Cyp1A1) och Aryl hydrocarbon-Receptorn (AhR) som svarar på exponering för organiska ämnen som t.ex. dioxin och olika PAHer. Glutation-S-transferas (Gst) indikerar ökad aktivitet för fasomvandling av främmande ämnen, catalase (Cat) och superoxiddismutas (Sod1, 2 och 3) svarar på fria radikaler och organiska metaboliter. Metallothionein (MT1a) svarar på metaller, stress och oxidativ stress.

Hos MCF7 celler analyserades Cyp1a1 samt Östrogenreceptor α (ERα) och östrogenreceptor β (ERβ). THP1 cellerna användes för analys av immunsvar på proteinnivå med IL-6 och TNF-α.

Analys av genuttryck hos HepG2 celler

HepG2 celler har sitt ursprung i leverceller och är därför väl lämpade för att testa toxicitet. Cellerna exponerades för de olika vattenproverna utan spädning. Exponeringen fortgick i 24 timmar varefter cellerna användes för extraktion av RNA.

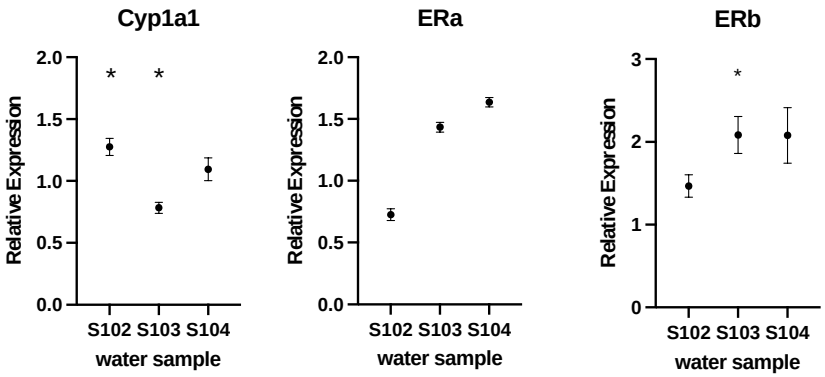


Figur 3. Genotox profile® analys av genuttryck hos HepG2 celler efter exponering för vatten från Strömsunds reningsverk.

För vatten från Strömsund föreligger ingen skillnad mellan ozonering och e-peroxone behandling jämfört med icke exponerad kontroll utom för Sod3 i S104 vilket kan reflektera en svagt ökad oxidativ eller närvaro av organiska metaboliteter. Statistisk analys redovisas som p<0,05 (*) eller p>0,01 (**).

Analys av genuttryck hos MCF7 celler

MCF7 cellerna kommer från en bröstcancer och reagerar på östrogena ämnen. Därför analyserades östrogenreceptor alfa (ERα) och östrogenreceptor beta (ERβ) i dessa celler.



Figur 4. Analys av genuttryck hos MCF7 celler efter exponering för vatten från Strömsunds reningsverk

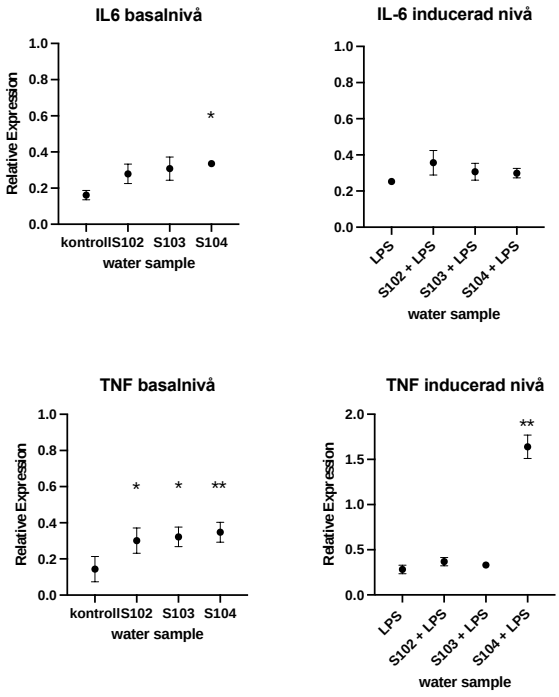
Resultaten visar att Cyp1a1 är svagt påverkat i S103 även om effekten är minimal. För östrogen signalering kan endast en liten statistiskt signifikant förändring iakttas efter ozonering i prov S103. Eftersom det råder små skillnader mellan de bägge behandlade prover och jämfört med obehandlat är det svårt att dra slutsatser kring närvaron av östrogena ämnen som förefaller låg utifrån utförd biotest. Inga östrogenliknande ämnen ingick i den screening för läkemedel som utfördes och analysdata för t.ex. 17-β *e*stradiol saknas.

Analys av immunsvar hos THP1 celler
För analyser av effekter på immunceller används THP1 celler. Cellerna behandlas med 10mM PMA för att omvandla cellerna till makrofager. Som positiv kontroll användes lipopolysaccharide (LPS).

Exponering av THP1 celler för de olika vattnen utan förinducering med LPS visar att IL6 basnivå är något förhöjd efter e-peroxone behandling av vatten (S104).

TNFα nivåerna påverkades i samtliga vatten från Strömsund. Här var en generell induktion av TNFα efter både ozonering och e-peroxone behandling. Efter LPS inducering kan man se en kraftigt ökad signal efter e-peroxone behandling (S104) vilket kan tolkas som att något ämne som stör immunsystemet via TNFα antingen frisätts från matrisen i obehandlat vatten alternativt formeras under oxidationsbehandling.

I övriga fall verkar störningen på immunsystemet generellt bero på komponenter i obehandlat vatten som behandling med avancerad oxidation inte kan reducera. Således verkar effekten ej bero kunna tillskrivas läkemedelsrester som reduceras vid behandling, men trots detta är skillnaden mot obehandlat vatten liten för IL6 och TNFα på basalnivå.



Figur 5. Analys av proteinnivåer för IL6 och TNFα i THP1 celler efter exponering för vatten från Strömsunds reningsverk. Effekter på både basnivåer och inducerade nivåer har testats.

BioImpakt AB

Utvärdering av resultat och slutsatser

En sammanvägd analys av resultaten visar att det kvarstår vissa effekter efter både ozonering och e-peroxone behandling. Akut toxicitet reduceras kraftigt genom behandling av både ozon och e-peroxone med minskning av mortalitet hos vattenloppa från obehandlat prover. Reduktionen gick från 55 % hos obehandlat vatten till ca 30 % för ozonering och 25 % hos e-peroxone.

Vid tolkning av utförda genanalyser och enskilda biomarkörer är det svårt att uttala säkra skillnader mellan obehandlat vatten och de två behandlingstekniker som jämförts. Avloppsvatten är komplext i sin natur och det finns fler ämnen och komponenter än endast läkemedelsrester som varit fokus i utförda analyser. Med tanke på de kraftigt minskade nivåerna av läkemedel så beror effekterna troligen på andra ingående föroreningar.

Örebro 20221228

För BioImpakt AB

Per-Erik Olsson
Projektledare

BILAGA 1

Tabell 1: Gener som ingår i Genotox Profile®-analysen presenterad i de föregående figurer.

Effekt	Förkortning	Fullständigt Namn	Genernas Funktion
Toxisk Respons	MT	Metallothionein	Metals and Free Radicals
	Hsp 60	Heat Shock Protein 60	General Stress Response
	Hsp 70	Heat Shock Protein 70	General Stress Response
	Hsp 90	Heat Shock Protein 90	General Stress Response
	Hsp 90b	Heat Shock Protein 90b	General Stress Response
	Gst	Glutathione-S-transferase	Conversion of Toxic Compounds
	Cat	Catalase	Reducing Free Radicals
	Dap 1	Death-associated Protein 1	Apoptosis
Reproduktion	Vtg 1	Vitelogenin 1	Egg Yolk Protein
	Vtg 2	Vitelogenin 2	Egg Yolk Protein
	Jhe	Juvenile Hormone Esterase	Juvenile Hormone Inactivation
	Cyp 314	Cytochrome P450 314	20-Hydroxyecdysone Synthesis
	Vmo 1	Vitelline Membrane Outer Layer 1	Oogenesis
	Ddx 4	Dead-box RNA Helicase 4	Germ Cell Development
	EcR A	Ecdysone Receptor A	Molting
	EcR B	Ecdysone Receptor B	Molting
	Ftz-F1	Fushi Tarazu Factor 1	Sex Differentiation
	E74	E74	Ecdysone Regulated
	E75	E75	Regulates Molting Cycles
	Usp	Ultraspiracle	Juvenile Hormone Receptor

Bilaga 4

I denna bilaga redovisas genomförda insatser som icke tekniska sammanfattningar gällande frågor om övergripande projektutfall, projektintern - och extern kommunikation, massmedial kommunikation, tidsplanering och förseningar, samt och projektutfall vad gäller kostnader. Redogörelse är linje med Naturvårdsverkets riktlinjer för bidragsfinansierade förstudieprojekt.

Måluppfyllnad och projektutfall sammanfattas i tabell 1 nedan.

Tabell 1.

Kriterier enligt projektbeskrivningen	**Måluppfyllelse i utfört förstudieprojekt**	**Kommentarer Strömsunds kommun**	**Kommentarer Envix**
<u>Tidplan för utförande av projekt:</u> Augusti 2021 till oktober 2022.	Nej, förseningar p.g.a. både pandemi- och krigsrelaterade faktorer	Längre leveranstid på komponenter gjorde att tidplanen inte kunde hållas. Vi har begärt längre projekttid hos NV.	Nytt system byggt och viss komponenter har fått längre leveranstider och ökade transporttider vilket är orsak till förseningar. Mer tid begärt vid 2 tillfällen.
Ekonomi/beviljade medel enligt ansökan: 3 957 000 sek	Nej, budget har överskridits med totalt 603 237 kr. Projekt har äskat mer pengar i augusti 2022. NV gav ej bifall till mer pengar. Se vidare specifikation under ekonomisk uppföljning nedan.	På grund av pandemi och världsläget har kostnader ökat rejält på ett sätt som ej gick att förutse när budget för projektet gjordes. Vi har ansökt om mer bidrag hos NV, men detta har ej beviljats.	Inköp av vissa komponenter för elektronik och PLC-styrning har kraftigt fördyrats p.g.a. brist under pandemin. Genom förseningar har även fördyringar skett genom forcering av byggnation som krävdes för att kunna genomföra projektet samt ökad grad av på plats-anpassning jämfört med budget

Måluppfyllning utifrån syftet med förstudien		**Kommentarer Strömsunds kommun**	**Kommentarer Envix**
Huvudsyftet: Att bedöma dagens rening med avseende på mikroföroreningar och utvärdera en ny avancerade oxidationsprocess, elektro-peroxone med jämförelse mot ozonering.	Ja, alla planerade tester under 4 årstider och varierande förhållanden har genomförts och all utvärdering och analys likaså.	Projektets syfte har varit att med stöd av beviljade medel utföra tester och utvärdera resultat på hur den avancerade tekniken fungerar på avloppsvatten som ska renas från läkemedel. Resultaten i den här förstudien i industriskala, visar att oxidationstekniken elektro-peroxone fungerar mycket bra och reningsgraden är högre än ozonering.	Utvärdering beskrivs ingående i huvudrapporten, men reningsgrad som kunde uppvisas med ny avancerad oxidationsprocess uppgår till nära 85 % i den utförda ”industriskalan”. Medelflödet låg strax över de 200 m^3/h som utlovats i projektplanen. Driftssäkerheten för byggd pilotanläggning och inbyggd automatik har fungerat väl utan betydande incidenter. Kommunens driftspersonal har varit till mycket god hjälp för att lösa praktiska frågor under drift och genomförandet.
Analysomfattning:			
<u>Kemisk analys:</u> Större screening av läkemedel ca 100 st och selektion av relevanta ämnen som behövs för att studera effektivitet av reningsmetod. I övrigt baskarakteristik för avloppsvatten.	Ja		Totalt har läkemedelsanalys utförts för ca 130 prover i projektet Ytterligare ett fåtal i förtester och under optimering samt baskarakteristik
<u>Biologisk analys:</u> Både akuta tester och relevanta effektbaserade in vitro tester för relevanta markörer och tillräcklig	Ja		Ja, tester har följt utvecklingen på området och ett anpassat urval utifrån budgetförutsättningar av effektbaserade in vivo och in vitro tester har genomförts och rapporterats i projektet.

känslighet och specificitet bör tillämpas i projektet			
Kommunikation			
En kommunikationsplan upprättades inför projektet i ansökan. Följande punkter fanns med i planen:			
- upprätta populärvetenskaplig sammanställning som publiceras på kommunens hemsida.	Ja, delvis, sammanfattningar har publicerats som pressrelease	I samband med uppstarten av projektet så lades information ut på hemsidan ”Kommunen testar avancerade teknik för att rena avloppsvattnet från läkemedel”.	En svensk sammanfattning ingår även i slutrapporten vars huvudrapport är på engelska
- Löpande kommunicera runt projekt på kommunens hemsida	Ja	Pressrelease initialt, löpande uppdateringar har gjorts och pressmeddelande inför studiebesök och redovisning av resultat.	Envix har försökt ge kommunen nödvändig information för deras kommunikationsarbete
- Arrangera ett större regionalt seminarium om projektet för branschaktörer	Nej, följer efter projektavslut	Redovisning av projektet planeras att ske i VA-nätverk i länet när det är slutrapporterat.	Envix är villiga att ha en dragning om projektet vid sådant arrangemang
Kommunikation i övrigt			
Projektmöten	Ja	Alla inblandade i projektet från kommunens sida har deltagit i regelbundna projektmöten där	Avstämningsmöten har hållits 1 gång i månaden där pågående och planerade aktiviteter delgivits information kring.

		uppnådda resultat redovisats. Detta har gett oss insyn i hur projektet fortskridit och hur tekniken fungerat. Vi har fått kunskap som vi inte skulle ha fått ta del av om vi inte drivit detta projekt.	Vidare har genomgång av senaste framkomna resultat i projekt skett vid dessa möten och det har funnits tillfälle för kommunen att ställa frågor. Dessa möten har hållits regelbundet och utgjort en viktig del i kontinuiteten i projekt och att alla berörda i projektorganisationen hållits informerade.
Studiebesök	Ja, hölls 17 nov 2022 Kommunanställda och politiker inbjöds till studiebesök	Till studiebesöket bjöds andra intresserade kommunanställda, politiker in och ett pressmeddelande gick ut inför studiebesöket.	Envix höll en populärvetenskaplig genomgång om projektet och framkomna resultat och hade en öppen frågestund kring projektet tillsammans med kommunen.
Mediakontakter	Ja, flera olika medier har uppmärksammats projektet. Troligen har media sett kommunens pressrelease och kommunikation via hemsidan om projektet.	Alla tidningar i Jämtlands län var närvarande vid studiebesök 17 november, liksom lokal-tv SVT Jämtland och SR Jämtland som båda gjorde ett reportage i samband med dagen där både Strömsunds kommun och Envix intervjuades om projektet. TV4 gjorde ett eget reportage i början av december 2022 som sändes på rikskanalen TV4 på nyårsafton. Tidningen cirkulation som är en branschtidning för VA-branschen gjorde ett reportage under	Envix har svarat på tekniska frågor som ställts vid samtliga mediakontakter och försökt beskriva projektets syfte och bakgrund på ett förståeligt sätt. Ett flertal mediakontakter har hållits under projektet som listas under kommunens kommentarer. Det är kul att responsen på projektet varit positivt både hos Strömsunds kommun och olika media. Det känns som att temat kring läkemedel i miljön nått ut brett vilket skapat både intresse och förståelse för att det är en prioriterad miljöfråga där nya lösningar

		december som publicerades 9 februari 2023. Även en norsk branschtidning har hört av sig och ville ta del av projektet. Det har tydliggjorts i samtliga fall att Naturvårdsverket är finansiär för förstudieprojektet.	efterfrågas vilket projektet är ett exempel på.
Sekretess		Förutom att alla deltagare i projektet skrev på sekretessavtal så sattes skyltar upp med ”fotografering förbjuden” kring utrustningen. Även en presenning sattes upp för att avskärma containern med utrustningen från övriga reningsverket.	Ett sekretessavtal skrevs med Strömsunds kommun med anledning av pågående ansökningsprocess runt immateriella rättigheter för nya avancerad oxidations -process. Detta har fungerat mycket bra och Strömsunds kommun har gjort allt för att respektera gränsdragningarna inom denna fråga. Avtalet var också bra då projektet kommunikation och fördjupade diskussioner kunde hållas vid projektmöten om utförda tester och resultat som framkommit med ökad förståelse för tekniken som tillämpats i projektet.
Ekonomisk uppföljning			
Beviljad budget	3 957 000 sek		
Betalplan	Ja	Betalplan upprättades och bifogades ansökan till NV. De	Envix föreslog en betalplan inför projektet med fasta tidpunkter för

		beviljade medlen räckte dock inte till de ökade kostnaderna som uppstod på grund av pandemi och världsläget.	reglering utefter som kostnader uppstod. Betalplanen var ”framtung” då merparten av kostnader för byggnation uppstod tidigt i projektet. Strömsunds kommun antog denna betalplan för reglering av Envix´s kostnader i projektet. Återstående del av beviljade medel som erhålls efter slutrapportering delades 50/50 mellan parter att ligga ute med under projektet senare faser.
Uppföljning och utfall	Ja, utförd. Budget överskreds med 603 237 sek.	Uppföljning av ekonomin har gjorts löpande i projektet. Tyvärr har fördyringar skett som vi inte kunnat råda över och detta har inneburit att budget har överskridits. Ansökan om mer medel på grund av fördyringarna har gjorts men NV har inte beviljat detta. Eftersom besked från NV kring mer bidrag för fördyringarna kommit väldigt sent (avslaget) innebar det att inget i projektplanen ändrades. Har beskedet om avslag kommit tidigare har en del justeringar varit möjliga att göra.	Löpande uppföljning på utgifter i förhållande till ursprunglig budget gjordes. Inköp av vissa komponenter för elektronik och PLC-styrning har kraftigt fördyrats p.g.a. brist under pandemin. Genom förseningar har även fördyringar skett genom forcering av byggnation som krävdes för att kunna genomföra projektet samt ökad grad av på plats-anpassning jämfört med budget. En framställan kring dessa merkostnader gjorde till Strömsunds kommun och vidare till Naturvårdsverket för att begära mer medel då projektparterna ej haft full rådighet över de fördyringar som skett. Naturvårdsverket sa nej till begäran om ytterligare medel. Envix vill

			framhålla att detta besked borde kommit långt mycket tidigare från Naturvårdsverket efter att begäran inlämnats då det funnits möjlighet att hushålla med projektmedlen bättre för slutfasen av projektet och ev. omvärderat omfattning av kvarstående arbeten. Nu genomfördes allt enligt projektplan och det fick en som konsekvens extra och icke förväntade merkostnader som både Envix och Strömsunds kommun drabbades av. Vid begäran p.g.a. merkostnader har redovisning redan skett av dessa medel.
Redovisning av kostnadsposter i projekt	Ja	Enligt specifikationer nedan. Utdrag från Strömsund kommuns ekonomisystem.	

Slutkommentarer från Strömsunds kommun: För vår del har detta varit ett mycket intressant projekt och har bidragit med kunskap kring läkemedelsrening som vi inte skulle haft möjlighet att ta del av om vi inte haft detta projekt. Det har varit intressant att se hur mycket bättre reningsgrad som kan uppnås med den testade tekniken, elektro-peroxone, jämfört med vår ordinarie teknik och även jämfört med ozonering. E-peroxontekniken kunde uppnå en hög rening av alla typer av mikroföroreningar, oavsett säsong och vattenkvalitet. Att i framtiden kunna investera i den här tekniken skulle vara en stor vinst för vattenmiljön i Ströms Vattudal.

Envix har varit mycket noga med att vi ska ha haft ett bra samarbete, de har haft personal på plats här i Strömsund i samband med installationen av utrustningen och provtagningen. Deras och vår driftpersonal på plats har tillsammans löst de oförutsedda utmaningar som dykt upp i samband med installationen av utrustningen på ett bra sätt.

Ekonomi

Projektutdrag följer nedan för förstudie ur Strömsunds kommun ekonomisystem

Proj
21020 Läkemedelsrening

PERIOD 2101-2302

INGÅENDE SALDO	
TRANS.SALDO	1.232.497,63
UTGÅENDE SALDO	1.232.497,63
ÅRSBUDGET	
VARAV TILLÄGGSBUDGET	
BUDGETAVVIKELSE	1.232.497,63-

PER	DATUM	VER.NR	TEXT	BELOPP	Kto	Ansv	Änd	Akt	Proj	Obj	Mp
2108	210813	2136863	Naturvårdsverket NV-21-006358	2.967.568,00-	351	14050	542		21020	85900	81
2109	210906	2154554	ENVIX	1.189.663,25	74513	14050	542		21020	85900	
2110	211006	2156524	ENVIX	1.231.744,75	74513	14050	542		21020	88500	
2111	211104	2158381	ENVIX	80.663,25	74513	14050	542		21020	88500	
2112	211231	2204714	Elektra i Jämtland AB	28.331,96	6153	14050	542		21020	88500	
2112	211202	2160390	ENVIX	278.504,75	74513	14050	542		21020	88500	
2112	211231	2161896	ENVIX	165.759,25	74513	14050	542		21020	88500	
2202	220207	2245858	ENVIX	382.123,25	74513	14050	542		21020	88500	
2203	220301	2246618	FÖLLINGE SVETS & MEK AB	37.627,12	6172	14050	542	280	21020	88500	
2204	220404	2249277	AHLSELLS AB	1.891,10	4102	14050	542		21020	88500	
2205	220501	2250966	Fortnox Finans AB	8.471,11	4103	14050	542		21020	88500	
2205	220517	2208056	Elektra i Jämtland AB	2.495,80	4104	14050	542		21020	88500	
2205	220523	2228614	Löner 2205, STR2205	11.836,36	50201	14050	542	960	21020	85900	
2205	220523	2228614	Löner 2205, STR2205	1.014,55	50208	14050	542	960	21020	88500	
2205	220523	2228614	Löner 2205, STR2205	225,45	50209	14050	542	960	21020	88500	
2205	220523	2228614	Löner 2205, STR2205	4.645,77	562	14050	542	960	21020	85900	98
2205	220523	2228614	Löner 2205, STR2205	486,70	562	14050	542	960	21020	88500	98
2205	220501	2250966	Fortnox Finans AB	12.160,00	6152	14050	542		21020	88500	
2205	220503	2251839	FÖLLINGE SVETS & MEK AB	88.453,62	6152	14050	542	280	21020	88500	
2205	220517	2208056	Elektra i Jämtland AB	4.809,00	6153	14050	542		21020	88500	
2205	220501	2250730	SWEDBANK FINANS AB/REAXER	29.956,87	74918	14050	542		21020	88500	
2206	220601	2252840	FÖLLINGE SVETS & MEK AB	18.511,12	6152	14050	542		21020	88500	
2206	220603	2254450	FÖLLINGE SVETS & MEK AB	7.108,04	6152	14050	542		21020	88500	
2207	220701	2255193	SWEDBANK FINANS AB/REAXER	927,25	74917	14050	542		21020	88500	
2208	220801	2257494	FÖLLINGE SVETS & MEK AB	10.204,00	6152	14050	542		21020	88500	
2208	220817	2258780	FÖLLINGE SVETS & MEK AB	2.087,25	6311	14050	542		21020	88500	
2212	221201	2263759	ENVIX	68.171,25	74513	14050	542		21020	88500	87
2212	221231	2269653	Envix Nord AB	603.236,75	74513	14050	542		21020	88500	87
2212	221231	2271595	Envix Nord AB	505.208,75	74513	14050	542		21020	88500	87
2212	221231	2271597	Envix Nord AB	603.236,75-	74513	14050	542		21020	88500	87
2212	221231	2271596	Envix Nord AB	54.918,50	74513	14050	542		21020	88500	87

Proj
21020 Läkemedelsrening

PERIOD 2101-2302

INGÅENDE SALDO	
TRANS.SALDO	1.232.497,63
UTGÅENDE SALDO	1.232.497,63
ÅRSBUDGET	
VARAV TILLÄGGSBUDGET	
BUDGETAVVIKELSE	1.232.497,63-

PER	DATUM	VER.NR	TEXT	BELOPP	Kto	Ansv	Änd	Akt	Proj	Obj	Mp
2212	221231	2343819	Envix Nord AB	48.602,43	74513	14050	542		21020	88500	87
2212	221231	2228714	Envix Nord AB Kreditfaktura 2022	76.536,87-	74513	14050	542		21020	88500	87
2301	230101	2326000	Envix Nord AB Kreditfaktura 2022	76.536,87	74513	14050	542		21020	88500	87
2301	230102	2343820	Envix Nord AB	76.536,87-	74513	14050	542		21020	88500	87

SLUT PÅ TRANSAKTIONER

Bokföringstransaktioner

Änd :542 Avloppsverksamhet

Proj :21020 Läkemedelsrening

Kto	Ansv	Änd	Akt	Proj	Obj	Mp	Datum	Vernr	Text	Belopp
351	14050	542		21020	85900	81	20210813	2136863	Naturvårdsverket NV-21-006358	- 2 967 568,00
74513	14050	542		21020	85900		20210906	2154554	ENVIX	1 189 663,25
74513	14050	542		21020	88500		20211006	2156524	ENVIX	1 231 744,75
74513	14050	542		21020	88500		20211104	2158381	ENVIX	80 663,25
6153	14050	542		21020	88500		20211231	2204714	Elektra i Jämtland AB	28 331,96
74513	14050	542		21020	88500		20211202	2160390	ENVIX	278 504,75
74513	14050	542		21020	88500		20211231	2161896	ENVIX	165 759,25
Summa										**7 099,21**

Bokföringstransaktioner

Änd :542 Avloppsverksamhet

Proj :21020 Läkemedelsrening

Kto	Ansv	Änd	Akt	Proj	Obj	Mp	Datum	Vernr	Text	Belopp
74513	14050	542		21020	88500		20220207	2245858	ENVIX	382 123,25
6172	14050	542	280	21020	88500		20220301	2246618	FÖLLINGE SVETS & MEK AB	37 627,12
4102	14050	542		21020	88500		20220404	2249277	AHLSELLS AB	1 891,10
4103	14050	542		21020	88500		20220501	2250966	Fortnox Finans AB	8 471,11
4104	14050	542		21020	88500		20220517	2208056	Elektra i Jämtland AB	2 495,80
50201	14050	542	960	21020	85900		20220523	2228614	Löner 2205, STR2205	11 836,36
50208	14050	542	960	21020	88500		20220523	2228614	Löner 2205, STR2205	1 014,55
50209	14050	542	960	21020	88500		20220523	2228614	Löner 2205, STR2205	225,45
562	14050	542	960	21020	85900	98	20220523	2228614	Löner 2205, STR2205	4 645,77
562	14050	542	960	21020	88500	98	20220523	2228614	Löner 2205, STR2205	486,70
6152	14050	542		21020	88500		20220501	2250966	Fortnox Finans AB	12 160,00
6152	14050	542	280	21020	88500		20220503	2251839	FÖLLINGE SVETS & MEK AB	88 453,62
6153	14050	542		21020	88500		20220517	2208056	Elektra i Jämtland AB	4 809,00
74918	14050	542		21020	88500		20220501	2250730	SWEDBANK FINANS AB/REAXER	29 956,87
6152	14050	542		21020	88500		20220601	2252840	FÖLLINGE SVETS & MEK AB	18 511,12
6152	14050	542		21020	88500		20220603	2254450	FÖLLINGE SVETS & MEK AB	7 108,04
74917	14050	542		21020	88500		20220701	2255193	SWEDBANK FINANS AB/REAXER	927,25
6152	14050	542		21020	88500		20220801	2257494	FÖLLINGE SVETS & MEK AB	10 204,00
6311	14050	542		21020	88500		20220817	2258780	FÖLLINGE SVETS & MEK AB	2 087,25
74513	14050	542		21020	88500	87	20221201	2263759	ENVIX	68 171,25
74513	14050	542		21020	88500	87	20221231	2269653	Envix Nord AB	603 236,75
74513	14050	542		21020	88500	87	20221231	2271595	Envix Nord AB	505 208,75
74513	14050	542		21020	88500	87	20221231	2271597	Envix Nord AB	- 603 236,75
74513	14050	542		21020	88500	87	20221231	2271596	Envix Nord AB	54 918,50
74513	14050	542		21020	88500	87	20221231	2343819	Envix Nord AB	48 602,43
74513	14050	542		21020	88500	87	20221231	2228714	Envix Nord AB Kreditfaktura 2022	- 76 536,87
Summa										**1 225 398,42**