Removal of Pharmaceuticals at Lycksele Sewage Treatment plant by an E-peroxone process

Results from a pilot-study financed by Naturvårdsverket

Final report

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SVENSK SAMMANFATTNING

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 läkedelsrester är således främst att förbättra skyddet den akvatiska miljön, men även för att skydda vatten som resurs t.ex. för dricksvattenproduktion och potentiellt skadlig exponering för människor.

Lycksele avfall och vatten AB (LAVAB) är ett kommunalt bolag i Lycksele kommun. LAVAB har varit projektägare för denna förstudie 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. LAVAB har bistått med teknikkunnande avseende konventionella reningsprocesser vid avloppsreningsverket i Lycksele samt personella resurser på plats och värdefull lokal kännedom om platsen. LAVABs kunskap om det aktuella avloppsvattnet och teknikstöd under etablerings- och anläggningsfas samt utförande har varit värdefullt för projektet. Förstudien har pågått under perioden augusti 2021 till februari 2023. Samtliga tester har utförts vid Lycksele avloppsreningsverk beläget i södra delen av Lycksele vid Forsbacka intill Umeälven som utgör recipient. Renat avloppsvatten släpps till älven via en ledning som mynnar ca 500 m nedströms Hällforsen kraftstation. Lycksele avloppsreningsverk är kommunens största och är dimensionerat för 14 000 pe med ett medelflöde om knappt 4000 m^3/dygn. Det har nyligen (2021) byggts ut och har idag en biologisk rening med MBBR-process.

Det övergripande syftet i projektet var att LAVAB tillsammans med Envix skulle implementera en ny avancerad oxidationsprocess, elektro-peroxon (E-peroxone) som tilläggsrening till konventionella reningssteg vid Lycksele avloppsreningsverk och i industriskala (215 m^3/24h) rena ett delflöde 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 detekterade 45 st vid något tillfälle i orenat avloppsvatten efter de konventionella reningsprocesserna. Baserat på medelvärden uppskattas ca 0,71 kg läkemedelsrester avrinna till recipienten varje dag vilket motsvarar ca 260 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 fördjupat bedöma risker för utsläpp av läkemedelsrester till Umeälven 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 90-95 % för alla säsonger. Metoden fungerade robust under alla förhållanden med mycket hög reningsgrad och hade i alla jämförelser högre reningsgrad än enbart ozonbehandling. Ingen bromatbildning kunde noteras varken vid E-peroxone eller ozonbehandling.

Den akuta toxiciteten sjönk väsentligt genom e-peroxonbehandling jämfört med enbart konventionellt behandlat avloppsvatten. 100 % mortalitet uppmättes i obehandlat vatten vilket reducerade till ca 10-15 % genom E-peroxonbehandling vilket innebär 85-90 % minskning i toxicitet. Även med ozonbehandling minskade som förväntat akut toxicitet, men reduktionen var inte lika stor och gick ner till 30 % (70 % reduktion). Vid analys av upp- och nedreglering av specifika gener som utgjorde biomarkörer för att påvisa subletala effekter uppvisade obehandlat avloppsvatten uppreglering av markörsgener som påvisar närvaro av dioxinlika eller andra främmande ämnen (t.ex. PAHer). Den effekten försvann helt efter behandling med E-peroxone och även med ozon vilket ytterligare underbyggde nyttan med behandlingen.

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 gathering 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 the 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 for 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 a recent mega survey by Wilkinson et al (2022), sampling was conducted in 258 rivers around all continents in 104 countries and that 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 Lycksele (LAVAB) Sewage treatment plant

A summary of the main STP at Forsbacka for Lycksele municipality is presented below. An overview of the process is also shown in Figure 1 and as Appendix 2.

The permit for the Lycksele STP allows treatment of up to 14 000 pe (population equivalents) and has a dimensioning flow (Q-dim) of 320 m^3/h. Lycksele STP receives sewage from all the central parts of Lycksele as well as suburban areas of Hedlunda and Lyckan. The main network of sewage and wastewater pipes within the Lycksele catchment areas are in total 103 km and includes 24 individual pump stations. Wastewater from the east side of Umeälven is collected at Stora Furuvik pump station from where wastewater is pumped through a submerged pipeline to the main plant at Forsbacka.

The processes in Lycksele STP consist of mechanical separation steps, biological treatment using MBBR-technique (Moving Bed Biofilm Reactor) as well as chemical treatment.

First step in the mechanical treatment is a rough separation through a larger mesh i.e., a stair screen, 3 mm in mesh size which is level controlled at the inlet of the screen. Screen reject is washed and compressed before discarded to a closed container with separate ventilation. After the mesh, the sewage passes an aerated sand filter. Separated sand in step 1 is pumped using mammoth pumps to a stainless steel drain along the sand filter which enters a pump well at the end of the filter. A submersible sand pump transports the sand/water suspension mix to a washing unit. Under normal operating conditions, the water that passes the aerated sand filter is led to a pre-sedimentation chamber, however it has a by-pass function enabling enter either to

the subsequent biological treatment or directly to the recipient. Fat residues and flotated solids from pre-sedimentation is pumped backed to inlet before the stair screen.

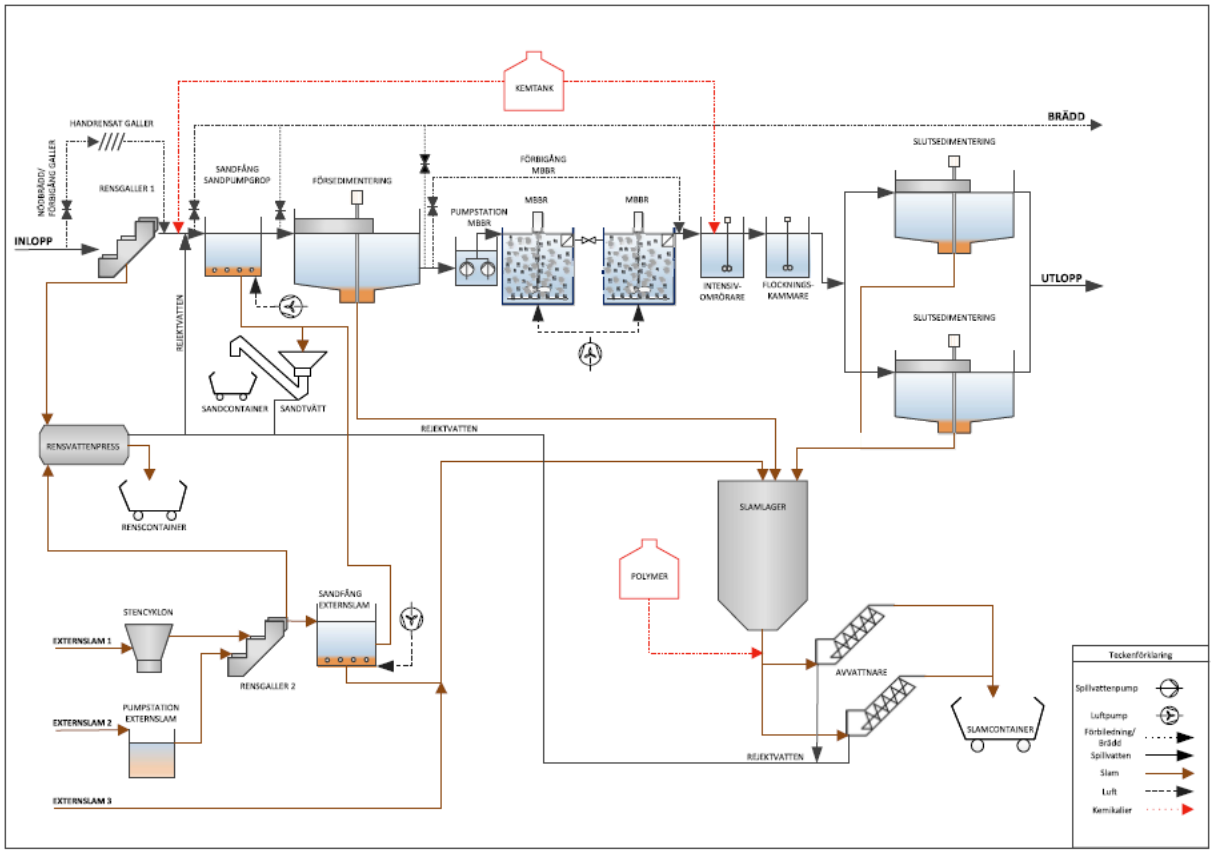


Figure 1. Process overview of Lycksele sewage treatment plan. (In Swedish). Source LAVAB.

Following pre-sedimentation, wastewater enters the biological treatment process which is two series of MBBR chambers. In order to sustain optimal growing conditions for the microbes, the water is continuously aerated using air blowers situated at the bottom of the chambers. The aeration is controlled to oxygen levels with known and adequate function. Plastic carriers in the bio-chambers facilitate and increase the growth of bacteria and overall removal efficiency.

Chemical treatment is performed by automated dosing of aluminum-based precipitation chemicals into a mixing container leading to a flocking container before entering the final and larger open sedimentation basins (two in parallel). The plant design also enables pre-precipitation into the wastewater directly after passing the stair screen and before the sand filter.

Separated and washed sand is discarded to a closed container. The sludge from both pre- and final-sedimentation, as well as other external sludge, is diverted to a sludge deposit from where actively pumped to two parallel dewatering units. At these units, a specific polymer (Zetag 8127) can be added to increase the dry content of the sludge up to approx. 15-30 % d.w. All generated sludge is disposed to waste facility for further recycling. Other external sludge entering the STP mostly occurs during summer time which can be screened through a mesh separately.

2.2 Site orientation and recipient

Lycksele STP at Forsbacka is situated in the southern part of the municipality of Lycksele, on the west shore of river Umeälven, see Figure 2. With its capacity of 14 000 pe, Lycksele STP is the largest treatment plant in the municipality. Its new biological process i.e., MBBR was introduced in 2021, see previous section for process description.

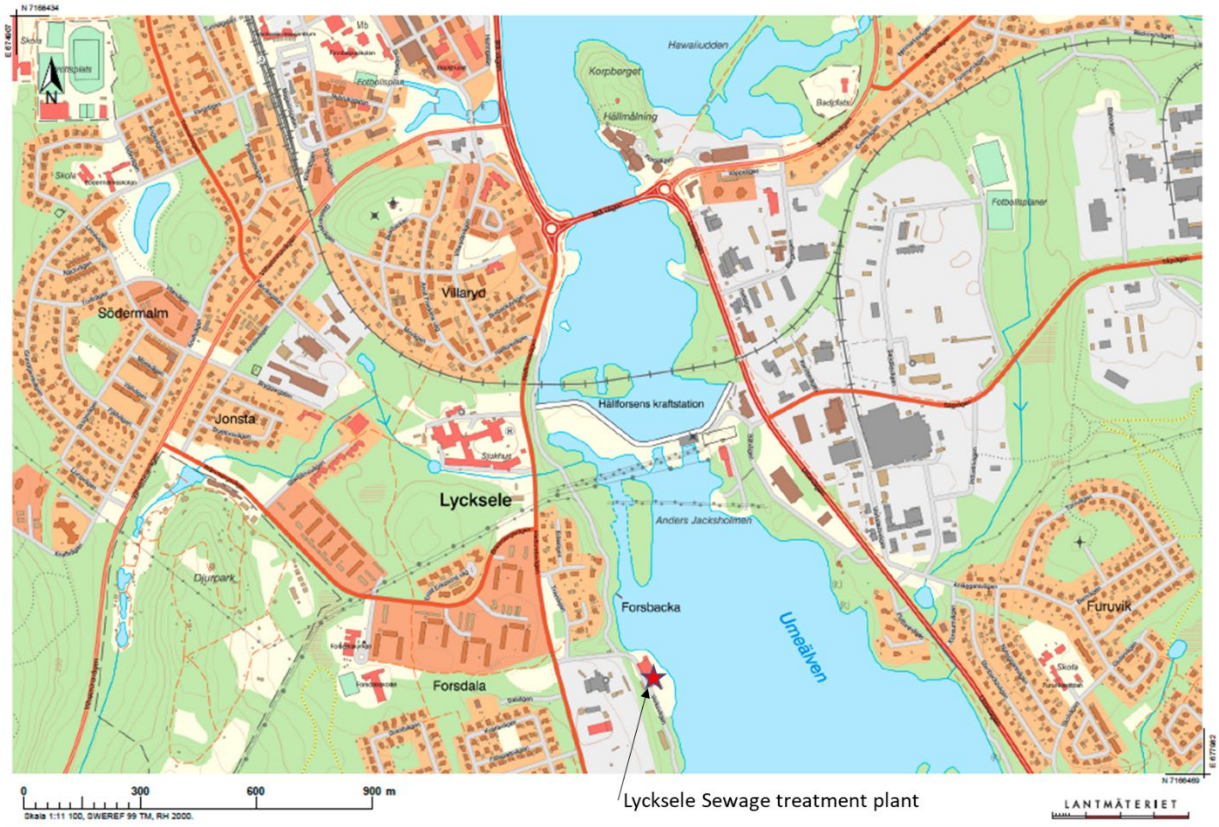


Figure 2. Location of Lycksele sewage treatment plant

The main recipient is river Umeälven, one of the largest rivers in Sweden. The emissions from Lycksele STP go to the stage of Umeälven called "Vattenförekomst Tuggens dämningsområde" in VISS[1] (water information system Sweden, Water ID WA36075502 / SE715545-164780). At this stage of Umeälven, the river has an average flow of 228 m^3/s according to the data from SMHI[2] (SUBID 27232, AROID 716466-163975).

Today this part of Umeälven has a poor ecological status, mainly due to the extensive hydropower with several dams and hydropower plants alongside the river. Umeälven is classified as heavily modified water body due to significantly affected hydrological regime or morphological condition. Good chemical surface water status prevails in the water body except for mercury and polybrominated diphenyl ethers (PBDEs), the latter a consequence of atmospheric deposition which is not caused by a nearby source and can not be attributed to local soruces i.e., sewage wastewater.

[1] https://viss.lansstyrelsen.se/Waters.aspx?waterMSCD=WA36075502

[2] https://vattenwebb.smhi.se/modelarea/

Up until 2021, analysis of pharmaceutical residues was missing for wastewater from Lycksele STP , however, some initial data was yielded within a masters degree project at Umeå University with the aim to increase knowledge on the issue.

The current quality criteria for this part of Umeälven is moderate ecological potential, hence lower quality criteria is required as compared to unmodified water bodies. However, within the topical criteria, viable populations of aquatic species must be maintained within the water body. Umeälven in Lycksele is popular for sport fishing of pike and perch, but also hosts populations of stream and migratory species such as grayling, trout and whitefish. The river area is thus important for recreational activities for Lycksele people and not just for fishing.

The water in Umeälven running through Lycksele municipality is the main recipient for the effluent from Lycksele STP, thus can potentially affect Umeälven downstream the STP. The recipient can therefore be considered important both for protection and preservation of aquatic life in the river but also to avoid the release of pharmaceuticals to the downstream catchment area of Umeälven. Further downstream from Lycksele, several smaller communities are located in the vicinity of the river, but also more populated areas, especially, Vännäs and Umeå municipalities, both situated along Umeälven before it mouths into the Bothnian bay at Obbola/Holmsund. Umeå city receives its drinking water from a groundwater aquifier close to Umeälven and a large amount of water from the river is infiltrated to increase formation of groundwater, subsequently used for drinking water production. Vännäs municipality has similar drinking water production and the future reservoir for Umeå is planned in the Vännäs area. This is important to mention since there are studies that have demonstrated pharmaceutical residues can occur in drinking water. However, in the risk assessment carried out by WHO regarding exposure via drinking water, pharmaceutical residues in drinking water are not deemed to pose any risk to human health at the levels analyzed in drinking water (World health organisation, 2012). Nonetheless, due to many remaining uncertainties related to e.g., bioaccumulating and persistent pharmaceuticals, it would be advised to study this matter further before excluding any risk that can arise from such emissions.

Arguably, protecting Umeälven is of general importance for a sustainable water cycle and health-safe water use in a longer time perspective. Sewage treatments targeting pharmaceutical residues is in principle important regardless of the recipient, as the uncertainties surrounding this substance group and its potential impact, in the event of release into the environment, are still great. Even in larger bodies of water such as Umeälven, which theoretically has a high degree of dilution (average flow at Lycksele 228 m^3/s, min flow approx. 34 m^3/s (SMHI vattenweb[3]), it is important not to contribute to the load of substances potentially hazardous to the environment and health. Alongside the river, there are also multiple other STPs and potential point sources of additional amounts of pharmaceutical residues that have the Umeälven as the primary recipient.

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 Lycksele, county of Västerbotten, Sweden. It is together with a twin project in Strömsund, Jämtland county, Sweden, the first pilot

[3] Swedish Meteorological and Hydrological Institute (SMHI) https://vattenwebb.smhi.se/modelarea/ data for main catchment area 28 Umeälven, sub-catchment area Rinner till Lill-Tannselet, SUBID 27232, AROID 716466-163975https://vattenwebb.smhi.se/modelarea/

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 an 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 treatment using only ozone has been performed to detect important differenses both in terms of removal efficiency of pharmaceuticals and regarding removal of toxicity. Overall, the Lycksele 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 Lycksele 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 FOR 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 Covid19 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 Lycksele and installed at Lycksele municipal STP beside the water treatment building as shown in Figure 3. To avoid freezing, the electrochemical cell for in situ generation of hydrogen peroxide and the treatment tank were installed in a separate room, built by Envix, beside 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 performace of ozone generator to generate ozone. The ozone production was regulated by the production power of ozone generator and the concentration of produced ozone was continuously monitored by an ozone analyzer. Gaseous ozone was injected in the water via diffusers installed in the bottom of the 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 secondary effluent (outgoing water) of Lycksele STP. The incoming water flow rate for the AOP pilot system was set at 9 m^3/h, corresponding to approx. 5% of the average flow of Lycksele STP, with a total hydraulic retention time of 30 minutes for AOP treatment unit. Lycksele STP provided an electricity connection point which was providing electricity to the mother electrical pannel of the pilot.

As a first step, ozonation process was optimized by optimizing the 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 evaluate micropollutants removal as well as toxicity removal of the treated water. The same optimized conditions were used to run the the E-peroxone process for different seasons.

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

5.1 Testing period and sampling

The test run of E-peroxone pilot was conducted during four seasons i.e., winter, autumn, summer and spring, almost for two weeks each season wih continuous run (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 04-15 and spring testing during May 12-19, 2022. The collected samples of test runs from all seasons were prepared on site immediately after collection and sent for micropollutants and toxicity analysis.

5.2 Analytical methods

5.2.1 Analysis of pharmaceuticals and benzotriazoles

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 analyzing 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 secondary effluent before and after E-peroxone

Lycksele STP effluent was characterized and compared with the treated effluent by E-peroxone and ozonation processes. The wastewater characterization results are presented in Table 2. The pH of STP effluent remained unchanged after ozonation whereas a sligh insignificant change was observed in E-peroxone treated effluent. Dissolved organic carbon (DOC) in the treated effluents increased almost 25% by ozonation and 16% by E-peroxone. Likewise, the total organic carbon (TOC) increased 14% each in the treated effluents by E-peroxone and ozonation. Increase in TOC content can be attributed to an increase in DOC levels. In addition, 9% higher increase in DOC level by ozonation than E-peroxone can be explained by the selective nature of ozone which reacts with the unsaturated moieties in DOC with very high rate.

The color of the secondary effluent decreased after E-peroxone treatment whereas no significant decrease in colour was seen after ozonation treatment. This is contrary to the results of the parallel project in Strömsund. One possible reason could be that some colour giving compounds or organic matter complexes could not be oxidized during ozonation treatment due to selective ozone oxidation. The turbidity of the effluent remained almost unchanged after ozonation however, increased almost 1.5 times after E-peroxone. No valid reason was found for this behavior.

The alkalinity of the secondary effluent decreased 15% by E-peroxone treatment but increased 8% by ozonation. The chemical oxygen demand (COD) remained almost unchanged after both processes. On the other hand, biochemical oxygen demand (BOD) increased by 3mg/L (23% increase) in the effluents after both processes. Phosphorus level in the secondary effluent decreased 33% after E-peroxone treatment while remained unchanged after ozonation. Both oxidation processes had no effect on the total nitrogen in the effluent. In general, both E-peroxone and ozonation processes showed varying effects for various parameters in this project as compared to Strömsund project. Long term testing is required to verify the changes in the treated effluents after the E-peroxone process.

Table 2. Water characterization parameters of Lycksele 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		7.9	7.9	8.3
Color (410 nm)	mg Pt/L	22	20	<5,0
Turbidity	FNU	2.9	2.6	3.9
Alkalinity	mg HCO_3/L	130	140	110
Dissolved organic carbon (DOC)	mg/L	12	15	14
Total organic carbon (TOC)	mg/L	14	16	16
Chemical oxygen demand (COD-Cr)	mg/L	34	36	36
Biochemical oxygen demand (BOD)	mg/L	13	16	16
Phosphorus (P)	mg/L	0.15	0.15	0.10
Total nitrogen	mg/L	22	23	23

6.2 Occurrence of micropollutants at Lycksele STP

In total, 100 micropollutants were screened during four seasons in Lycksele STP effluent. Every season, approx. 2 samples were analyzed with screening of all 100 micropollutants. Out of 100, 45 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 Lycksele STP effluent were belonging to antidepressant class (8 compounds) followed by antibiotic and hypertension classes (7 pharmaceuticals in each class). After that, the other detected pharmaceuticals were belonging to psycholeptic (4), analgesic (4), antihistamine (3) and statin (2) classes. Both triazoles i.e., benzotriazole and methyl-benzotriazole were also found in the effluents. Few pharmaceuticals from other classes that are used for specific treatments, were also detected in Lycksele STP effluent such as anti-inflamatory, antiepileptic, antiarrhythmic etc.

Out of 45, the detection frequency of 29 micropollutants was 100%, 9 micropollutants between 50-100% while 7 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., 116 µg/L while lowest average concentration was found for clemastine i.e., 4 ng/L in Lycksele STP effluent. After caffeine, analgesic and antipyretic drug paracetamol had the highest concentration at around 47 µg/L. Interestingly, benzotriazole and methyl-benzotrizole were detected at relatively high concentrations, 2536 ng/L and 1761 ng/L, respectively. This is similar to the previous study where micropollutants were detected at 11 different STPs in Sweden (Lycksele not included), and benzotriazoles were the most common compounds in the effluents at lower concentrations 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.

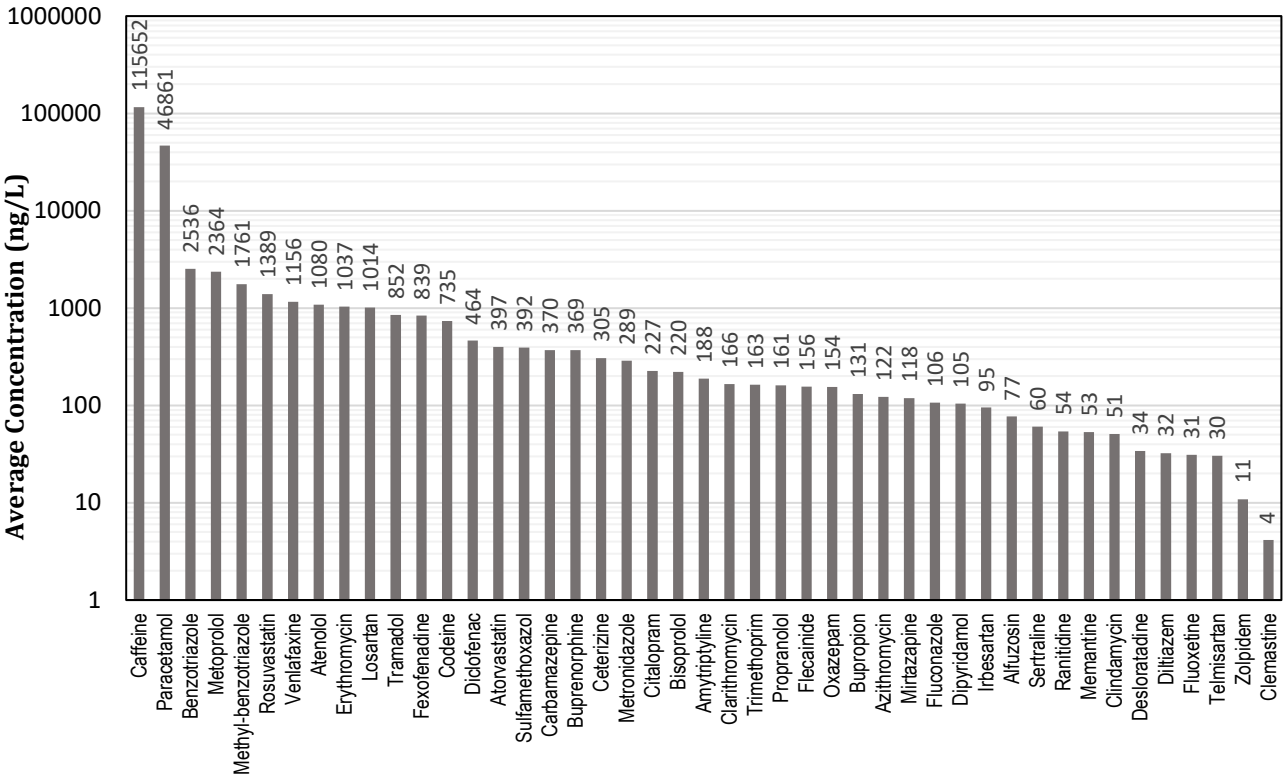


Figure 4. Occurrence of micropollutants in Lycksele sewage treatment plant effluent.

Out of 45 detected micropollutants in Lycksele STP effluent, 12 marker compounds 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 different 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 levels of these compounds for all four seasons are presented in Table 3 with their detection frequency.

All marker micropollutants were detected with 100% frequency during all seasons except for summer season. The detection frequency for diclofenac, memantine and oxazepam was 80% during summer whereas 100% for other marker micropollutants, similar to other seasons. The overall dectection frequency of marker compounds are significantly higher in Lycksele STP effluent as compared to Strömsund STP effluent.

Among marker micropollutants, highest variation in concentrations were observed for paracetamol, ranging from 17471 to 105062 ng/L. For paracetamol, highest levels were detected during spring season while lowest levels were detected in summer. Carbamazepine and methyl-benzotriazole were detected at their highest concentrations during winter season while low variation in concentrations were seen for other seasons. For benzotriazole, substantially higher concentrations were observed during summer (7764 ng/L) as compared to rest of the seasons (in the range of 670-1047 ng/L). In contrast, all other marker micropollutants were found at lower level and their average concentrations did not vary substantially over different seasons.

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

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 (04–15 July, 2022)**					**Spring Season (12–19 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]	424	1047	762	208	100	565	1256	948	302	100	540	30201	7764	12821	100	621	720	670	–	100
Carbamazepin	3×10^{5} [b]	8.8×10^{9} [b]	285	1263	606	387	100	279	444	332	64	100	160	346	286	78	100	223	287	255	–	100
Citalopram	1.1×10^{3} [c]		227	415	292	67	100	252	409	335	63	100	72	207	119	60	100	117	203	160	–	100
Diclofenac	1×10^{6} [b]	7.5×10^{9} [b]	272	677	446	154	100	319	1946	819	598	100	<LOQ	900	356	378	80	171	301	236	–	100
Fluconazole	<1 [a]	4.6×10^{9} [a]	88	188	116	36	100	86	288	182	80	100	26	104	77	31	100	41	60	51	–	100
Irbesartan	24 [d]	10^{10} [d]	37	280	111	91	100	37	111	72	27	100	96	195	150	43	100	42	51	47	–	100
Memantine	7.75 [c]		40	94	63	23	100	49	97	74	16	100	<LOQ	56	32	16	80	40	45	42	–	100
Methyl-benzotriazole	780 [a]	8.6×10^{9} [a]	1024	6476	3870	2018	100	602	2231	1367	606	100	373	1408	760	404	100	909	1184	1046	–	100
Metoprolol	2.0×10^{3} [a]	7.3×10^{9} [e]	2087	3038	2611	427	100	2288	3687	2952	630	100	1755	2051	1912	120	100	1492	2468	1980	–	100
Oxazepam	~1 [a]	9.1×10^{9} [a]	108	235	162	45	100	140	258	189	43	100	<LOQ	191	136	50	80	114	149	131	–	100
Paracetamol	2.57×10^{6} [f]		22070	71600	38357	18663	100	16286	43880	26554	9815	100	11980	24825	17471	5121	100	93741	116383	105062	–	100
Trimethoprim	2.7×10^{5} [a]	6.9×10^{9} [g]	121	286	197	56	100	143	366	248	88	100	43	79	64	14	100	128	155	142	–	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)

6.3 Mass flow of micropollutants

The total mass flow of the micropollutants per day, based on 100 screened compounds, were calculated in Lycksele STP effluent for each season and presented in Table 4. Variations in mass flow can be seen for different seasons. The larget mass flow of 0.94 kg/day was observed for autumn season followed by spring season with 0.88 kg/day. Interestingly, lowest mass flow was found for winter season, i.e., 0.46 kg/day. Note that the mass flow is based on average daily wastewater flow i.e., 3947 m^3/day at Lycksele STP for 2022. The average mass flow of all four seasons indicates that 0.71 kg of these micropollutants (from 100 screened micropollutants) are being released every day by Lycksele STP into Umeälven. By expanding the mass flow for the whole year with average flow, Lycksele STP is releasing ~260 kg of (mainly) pharmaceuticals mass into the environment (Umeälven) with its current conventional treatment processes. It is worth mentioning that this number i.e., 260 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 released into the environment by Lycksele STP is expected to be much higher.

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

Season	**Mass flow of micropollutants (kg/day)**
Winter season	0.46
Autumn Season	0.94
Summer Season	0.57
Spring Season	0.88
Average	0.71

6.4 Micropollutants removal during ozonation and optimization of ozone dose

Lycksele STP effluent was treated at three different ozone concentrations for micropollutants removal by ozonation. These concentrations correspond to specific ozone doses (SODs) of 0.4 gO_3/gDOC, 0.65 gO_3/gDOC and 0.9 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 ozone-reactive (O_3-reactive) micropollutants i.e. diclofenac, paracetalmol and trimethoprim ($k_{O3} > 10^4$ $M^{-1}s^{-1}$) were almost completely

removed at SOD of 0.4 g O_3/gDOC during ozonation except for carbamazepine which was removed to 88%. The complete (or very high) removal of these micropollutants can be attributed to their high k_{O3} values (Lee et al., 2014). Since paracetamol, diclofenac and trimethprime were either completely removed or showed very high removal at 0.4 gO_3/gDOC, further increase in SOD to 0.65 and 0.9 gO_3/gDOC did not affect the removal of these compounds. Interestingly, carbamazepine removal did not improve by further increase in SOD. In general, these results agree with previous studies where SODs of 0.5 gO_3/gDOC (Lee et al., 2014) and 0.47 gO_3/gDOC (Hollender et al., 2009) has been reported 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 quick reaction with ozone even at low SODs. This is why, the direct reaction of ozone accounts for 82% of the removal of O_3-reactive micropollutants while •OH mediated removal explaining the remaining 18% (Lee et al., 2013).

Removal of moderately O_3-reactive micropollutants: Of the four 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. All micropollutants in this group removed poorly at 0.4 gO_3/gDOC, ranging 29-52% 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 compounds. Lee et al. (2013) reported that an additional source of •OH (Lee et al., 2013) or higher ozone dose is required for their better removal especially for micropollutants with low k_{O3}.

Further, an increase in SOD to 0.65 gO_3/gDOC resulted decrease in removal of all moderately O_3-reactive micropollutants. In fact, the concentration of methyl-benzotriazole increased significantly after treatment (refered as negative removal) by increasing the SOD to 0.65 gO_3/gDOC which is shown as negative removal in Figure 5.

Negative removal has been observed previously, and has been associated with sorption of micropollutants to particles, which would consequently protect these micropollutants from oxidation (Huber et al., 2005; Mustafa et al., 2020; Zimmermann et al., 2011). During treatment, these micropollutants may desorb from particles, and this would explain the observed increases in concentrations. Depending on the oxidation kinetics, the released micropollutants might be oxidized further. In some cases, negative removal at wastewater treatment plants has also been attributed to the conjugation and de-conjugation of micropollutants (Vieno et al., 2007; Zorita et al., 2009).

Further increase in SOD to 0.9 gO_3/gDOC resulted significant improvement in removal for all moderately O_3-reactive micropollutants. For instance, metoprolol removed completely (100%) with an improved removal of 70%. The removal of citalopram and benzotriazole increased to 80% and 85%, respectively. Similarly, benzotriazole removal increased by 51%, however, still showed relatively low removal i.e., 65% at 0.9 gO_3/gDOC in comparison to other micropollutants in this group. 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).

Removal of O_3-resistant micropollutants: O_3-resistant micropollutants ($k_{O3} < 10^2$ $M^{-1}s^{-1}$) also showed low removal at 0.4 gO_3/gDOC in the range of 9-53%. Likewise, increase in concentrations (negative removal) were seen again for memantine and fluconazole when SOD increased to 0.65 gO_3/gDOC. Irbesartan and oxazepam were removed 27% and 22%, repectively at this SOD which is even lower removal than at 0.4 gO_3/gDOC.

By increasing SOD to 0.9 gO_3/gDOC, the removal of all O_3-resistant micropollutants improved substantially in the range of 45-72%, memantine with highest removal while oxazepam with lowest removal. The reason for relatively low removal of O_3-resistant micropollutants is because

they are predominantly oxidized by •OH (Katsoyiannis et al., 2011) due to low ozone reactivity and are often only marginally removed by ozone from wastewater. The is due to the fact that formation of •OH during ozonation depends on the water matrix, and is often slow in relation to hydraulic residence (Von Sonntag and von Gunten, 2012). In general, the removal of O_3-resistant micropollutants during ozonation in this project is slightly higher than previous results. The possible reasons for this behavior could be longer hydraulic residence time in comparison to other studies.

Due to optimal removal of micropollutants, SOD of 0.9 gO_3/gDOC was chosen for rest of the project for optimization of E-peroxone process and testing during different seasons.

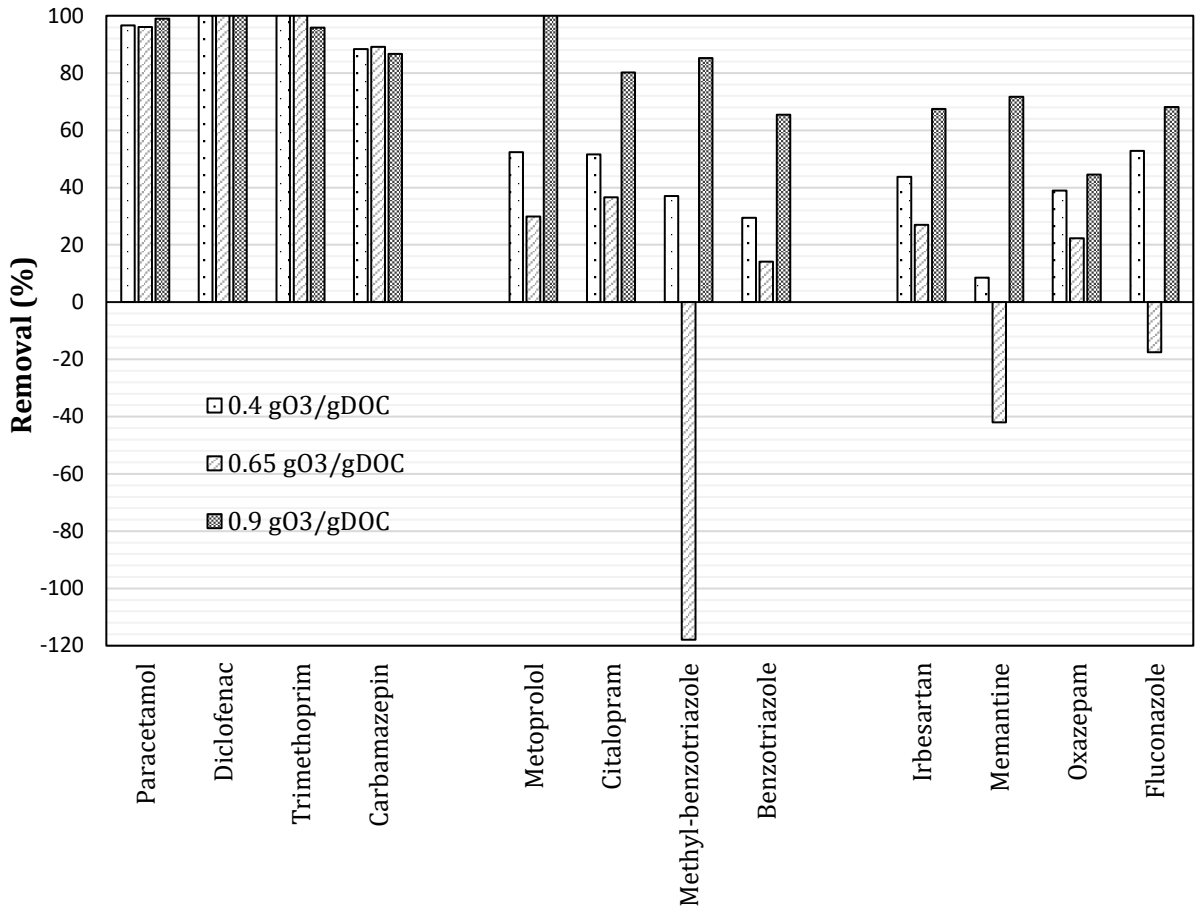


Figure 5. Removal (in %) of micropollutants in Lycksele secondary wastewater effluent during ozonation with SODs of 0.4, 0.65 and 0.9 gO_3/gDOC.

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

Conventional ozonation showed incomplete removal for few moderate O_3-reactive and lower removal for O_3-resistant micropollutants. Among tested SODs, 0.9 gO_3/gDOC appeared to be the optimal dose for removing micropollutants. Thus, this ozone dose was used for optimization of E-peroxone process. The removal of micropollutants by the E-peroxone process in comparison to ozonation is illustrated in Figure 6. It is important to mention that same ozone dose was used during both processes and the ratio of ozone and hydrogen peroxide i.e., $[O_3]$:$[H_2O_2]$ was set at ~1.2 for the E-peroxone process.

As expected, the E-peroxone process had no effect on the removal of O_3-reactive compounds. In fact, these micropollutants are supposed to be removed by ozonation. These micropollutants, with very high ozone reactivity, are removed quickly even at very low ozone dose. Thus, it can be concluded that the removal results of O_3-reactive micropollutants, shown in Figure 6, presenting their removal predominantly by ozonation rather than by the E-peroxone process.

Further, E-peroxone process showed varying effect on removal of micropollutants that had moderate reactivity with ozone. For instance, metoprolol removal decreased 20% by E-peroxone process than from ozonation, and yet achieving high removal of 80%. This decrease in removal can be attributed to metoprolol relatively high second order rate constant in moderately O_3-reactive group. By switching to E-peroxone process from ozonation, the selective oxidation of metoprolol by ozone is slightly decreased due to the parallel reaction of ozone with electrochemically generated hydrogen peroxide. The removal of citalopram and methyl-benzotriazole increased only by 9% and 4% by E-peroxone, resulting 89% removal for each compound. In contrast, E-peroxone process improved the removal of benzotriazole to 81% with an increase of 16% from ozonation. The reason for higher improvement in removal of benzotriazole by E-peroxone than other moderately O_3-reactive micropollutants is because it 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, removal of benzotriazole by oxidation increased.

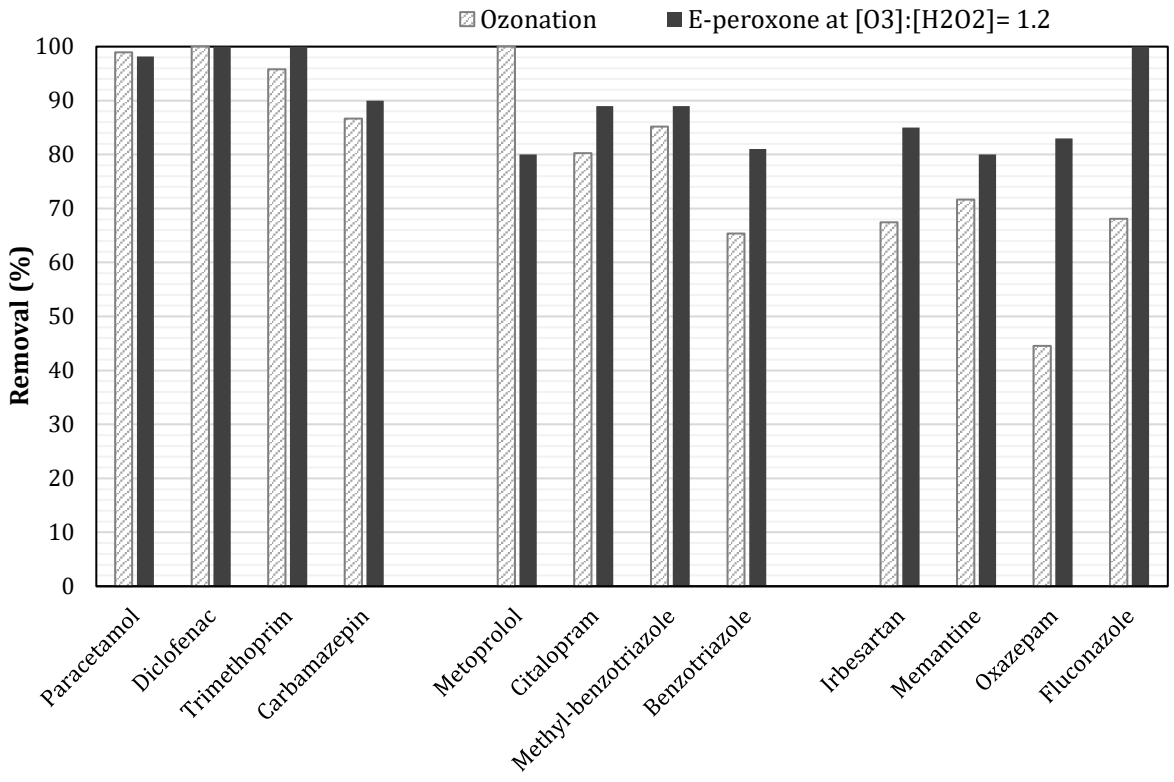


Figure 6: Removal (in %) of micropollutants in Lycksele secondary wastewater effluent during E-peroxone process at $[O_3]:[H_2O_2]$=~1.2 in comparison to conventional ozonation at 0.9 $gO_3/gDOC$.

As expected, the E-peroxone process improved the removal of all O_3-resistant micropollutants in comparison to ozonation. The E-peroxone process was able to remove 85% of irbesartan, 80% of memantine, 83% of oxazepam, and complete removal (100%) of fluconazole. This represent

improvements of 18% for irbesartan, 8% for memantine, 38% for oxazepam and 32% for fluconazole by E-peroxone process as compared to ozonation. These results contradict with previous study which reported E-peroxone process only marginally improves (< 10%) removal of micropollutants in secondary wastewater effluent 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 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 wastewater 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).

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 except for spring. During spring season, the pilot was runing mainly for optimization of both processes, i.e., E-peroxone process and ozonation. The purpose of testing the pilot 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 three seasons (winter, autumn and summer) testings are presented in Table 5 to 7. 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

Winter testing was conducted during the period of 14/11/2022 to 25/11/2022 with continuous run of the pilot system. The treated water samples were collected after almost 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. For comparison, conventional ozonation treated water samples were collected during the same time period and the average removal is presented in Table 5.

In general, the results followed the same pattern described in previos section 6.5 where all marker O_3-reactive micropollutants were removed either completely or showed very high removal in all samples. Among moderately O_3-reactive micropollutants, citalopram also showed very high average removal (>90%) by both processes. Metoprolol and methyl-benzotriazole were also removed efficiently for most of the samples by E-peroxone. Slightly low average removal of these compounds can be connected to negative removals in one sample for each compund. Excluding the removal of these particular samples with negative removal, the average removal improves substantially to 77% for methyl-benzotriazole and 73% for metoprolol. Both processes showed similar removal efficieny for these compounds. In moderately O_3-reactive group, lowest average removal was seen for benzotriazole, i.e., 44% by ozonation and 53% by E-peroxone.

The removal of O_3-resistant micropollutants was low for both processes in comparison to micropollutants from other groups and removal in other seasons. Among them, memantine and fluconazole showed lowest removal while highest removal was achieved for irbesartan during both processes. The improvement in removal of O_3-resistant micropollutants by E-peroxone process as compared to ozonation was increasing with the decrease of second order rate constants of micropollutants. An increase of 7%, 17%, 15% and 27% in removal was achieved by E-peroxone as compared to ozonation for irbesartan, memantine, oxazepam and fluconazole, respectively.

Table 5. Average removal of marker micropollutants in Lycksele 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	95	98	94	100	100	97	98	96
Diclofenac	95	100	100	100	100	100	100	100
Trimethoprim	100	100	100	100	100	100	100	100
Carbamazepine	99	99	94	100	100	100	100	100
Moderate O_3-reactive group								
Metoprolol	78	59 (73)*	100	63	−10	61	81	60
Citalopram	93	92	100	100	52	100	100	100
Methyl-benzotriazole	67	63 (77)*	100	89	41	−7	85	72
Benzotriazole	44	53	80	62	20	44	58	55
O_3-resistant group								
Irbesartan	56	63	92	95	43	25	47	77
Memantine	21	38	78	62	21	30	5	31
Oxazepam	46	61	64	69	54	68	58	54
Fluconazole	13	40	100	47	20	21	38	12

* The values presented in parentheses is the average removal without the samples of negative removal.

From full screening of 100 micropollutants, 33 micropollutants were detected during the winter testing. The removal results of all 33 micropollutants by E-peroxone process for the sample collected on 14/11/2022 is presented in Table S1. As can be seen, very high removal for almost all detected micropollutants was achieved except for few compounds. The lowest removal was observed for oxazepam (64%). The average removal of all detected micropollutants was 95% which is well above the requirement of 80% reduction, recommended by authorities and is considered acceptable.

6.6.2 Autumn Testing

Autumn testing was conducted during the period of 12/09/2022 till 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 micropollutants for individual samples and the average removal by E-peroxone is presented in Table 6.

The removal of O_3-reactive compounds showed similar results to winter testing that all micropollutants from this group were removed almost completely. The average removal of each micropollutant in this group was ≥95%. Among moderately O_3-reactive micropollutants,

citalopram, methyl-benzotriazole and metoprolol showed high average removals i.e., 92%, 81%, 76%, respectively. Benzotriazole showed slightly low removal (60%) which is mainly due to low removal for two samples (i.e., 36% each) collected on 12/09 and 14/09.

Table 6. Removal of marker micropollutants in Lycksele 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	98	98	97	99	97	100	100
Diclofenac	100	100	100	100	100	100	100
Trimethoprim	100	100	100	100	100	100	100
Carbamazepine	95	100	96	94	92	95	96
Moderate O_3-reactive group							
Metoprolol	76	71	70	77	92	76	68
Citalopram	92	86	92	100	93	88	93
Methyl-benzotriazole	81	100	60	81	88	83	71
Benzotriazole	60	36	36	71	77	78	64
O_3-resistant group							
Irbesartan	76	54	57	92	72	78	100
Memantine	77	70	76	100	81	60	78
Oxazepam	82	90	91	74	78	90	69
Fluconazole	53	88	38	27	79	32	55

The removal of all marker O_3-resistant micropollutants by E-peroxone was significantly higher for autumn testing than winter testing. Irbesartan, memantine and oxazepam showed average removal of 76%, 77% and 82%, respectively. These removals are significantly higher than winter results. Among O_3-resistant micropollutants, fluconazole showed lowest removal of 53%, yet 13% more than winter resuts.

The E-peroxone process and ozonation were compared again for removal of micropollutants for a sample taken 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 irbesartan and benzotriazole by 34% and 71%, respectively. Interestingly, the improvement in removal of memantine and oxazepam by E-peroxone is negligible (5% for both) as compared to ozonation. In contrast, the removal of metoprolol and benzotriazole is 25% and 24% higher for ozonation than E-peroxone. Similarly a slightly higher removal (8% more) was seen for citalopram during ozonation in comparison to E-peroxone. Interestingly, E-peroxone process improved the removal of O_3-reactive diclofenac to complete removal while it was removed 66% during

ozonation, with an increase of 36%. For other O_3-reactive compounds, similar removals were seen during both processes.

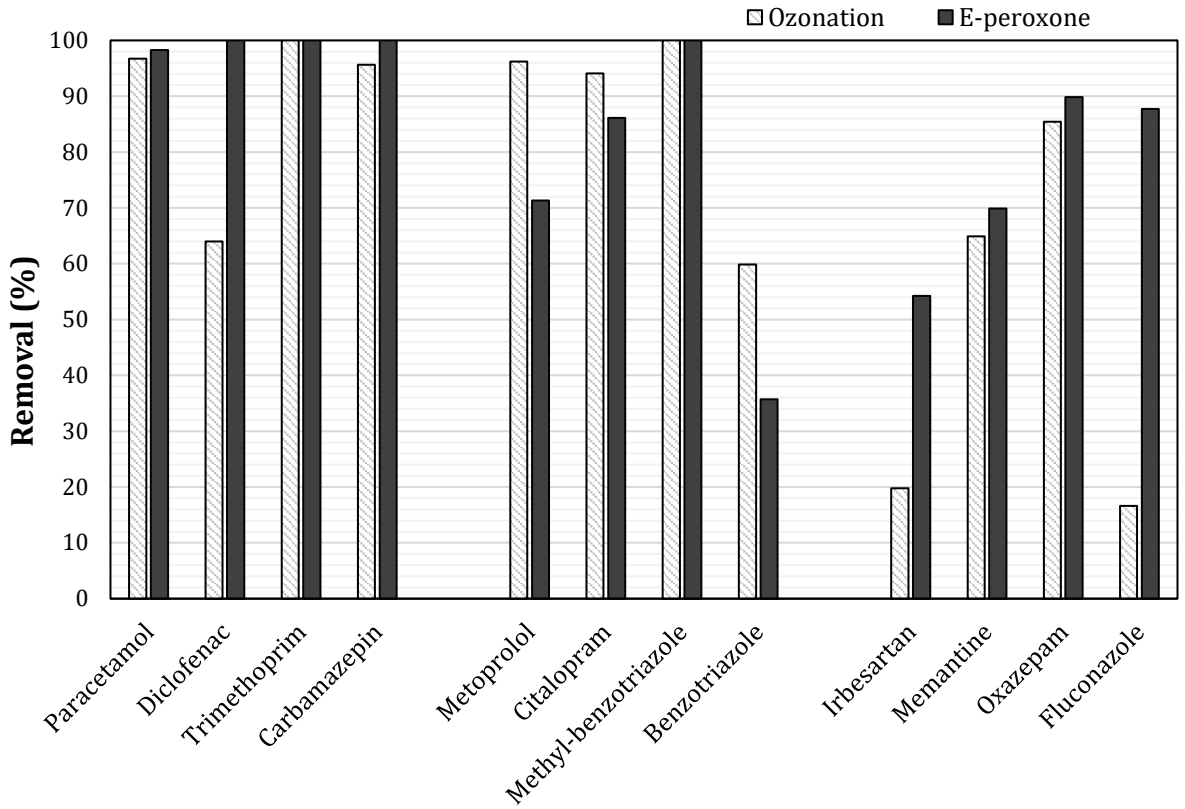


Figure 7. Removal of marker micropollutants by E-peroxone and ozonation in Lycksele STP effluent for the sample collected on 12/09/2022.

From full screening of 100 micropollutants, 34 micropollutants were detected during the autumn testing and the removal results of these 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 alfuzosin and benzotriazole (34% and 36%, respectively). Out of 34 detected, only 5 micropollutants were removed less than 80% while all other micropollutants were removed >80%. The average removal of all detected micropollutants was 90% which is, similar to winter results, well above the recommendation of 80% reduction.

6.6.3 Summer Testing

Summer testing was conducted during the period of 04/07/2022 till 15/07/2022 with the continuous run of the pilot system. The 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 completely or >90% except for trimethoprim (83% removal). Slightly low removal of trimethoprim in comparison to other compounds in this group is resulted from its low removal of sample 04/07 and 15/07. Among moderately O_3-reactive micropollutants, citalopram was completely removed. Metoprolol and methyl-benzotriazole also showed high removal of 85% and 81%, respectively. Similar to previous

seasons, benzotriazole was least removed at 53% mainly due to negative removal of sample 13/03. Benzotriazole removal was higher than 80% for all other samples except for this sample.

Table 6. Removal of marker micropollutants in Lycksele 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*				
		04/07	*08/07*	*11/07*	*13/07*	*15/07*
O_3-reactive group						
Paracetamol	100	100	100	100	100	100
Diclofenac	94	100	100	100	ND	76
Trimethoprim	83	63	100	100	100	52
Carbamazepine	93	96	100	76	93	100
Moderate O_3-reactive group						
Metoprolol	85	95	71	79	94	84
Citalopram	100	100	100	100	100	100
Methyl-benzotriazole	81	85	89	100	32	100
Benzotriazole	53 (86)*	89	81	89	−80	86
O_3-resistant group						
Irbesartan	95	89	100	89	100	100
Memantine	95	84	ND	95	100	100
Oxazepam	100	100	100	100	ND	100
Fluconazole	63 (80)*	63	78	78	−3	100

ND: Not detected

* The values presented in parentheses is the average removal without the sample 13/07.

The removal of marker O_3-resistant micropollutants was very high in comparison to winter and autumn testing except for fluconazole. For example, average removal of irbesartan, memantine and oxazepam was 95%, 95% and 100%, respectively by E-peroxone process. Fluconazole was also removed effectively for most of the sample except for the sample with negative removal on 13/07 which cause averge removal of 63% while the averge removal was 80% excluding this sample. For summer season, no ozonation sample was collected.

From full screening of 100 micropollutants, 36 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 was achieved. The lowest removal was observed for desloratadine (2%), an O_3-reactive compound. Out of 36 detected, only 3 micropollutants were removed less than 80% while all other micropollutants were removed >80%. The average removal of all detected micropollutants was 93% 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 was kept short from 12/05/2022 till 19/05/2022. The treated water samples were collected at different time points during the testing period under different consitions.

The two week spring testing with countious run at optimized conditions of E-peroxone could not be conducted because of the delay in installation caused by heavy snow and freezing conditions. As a result, an insulated room had to built for the electrochemical cell and the treatment tank. Thus, optimization of ozone dose and the E-peroxone process optimization were conducted late spring and the pilot was running continuously only during the optimization of these processes. The results of these testings have already been discussed in section 6.4 and 6.5.

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 •OH via a 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., 10 µg/L in drinking water (Von Gunten, 2003) and 50 µg/L according to the freshwater environmental quality standard (Soltermann, et al. 2016). During ozonation, one proposed method of minimizing bromate formation 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., 0.4, 0.65 and 0.9 gO_3/gDOC during ozonation treatment of Lycksele wastewater effluent, although the bromide level in Lycksele STP effluent was detected at 150 µg/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 results 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, L69) 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.

Very high acute toxicity in *Daphnia magna* was detected for the untreated effluent L69 with 100 % mortality in the highest exposure (100 % effluent). A large reduction in acute toxicity is seen for the treated effluents by ozonation (L70) and E-peroxone (L71 and L80) where acute toxicity is reduced to 30% after ozonation (L70) and 10-15 % after e-peroxone treatment (L71 and L80). Reduction of acute toxicity were about 70 % for ozonation (L70) and more than 85 % for E-peroxone treatment (L71, L80). The results are shown in Figure 8.

In Figure 8, corresponding acute test are also shown for Strömsund STP where the same tests were run and the outcome show a similar trend, however Strömsund had lower acute toxicity in untreated effluent. This supports the conclusion on that treatemnt with advanced oxidation considerably lowers the toxic effects after treatment. In Lycksele case, levels of pharmaceuticals were about 3 times higher compared to Strömsund STP which also suggest that pharmaceuticals potentially could have contributed to the detected acute effects since the acute effects were higher for Lycksele samples as compared to Strömsund.

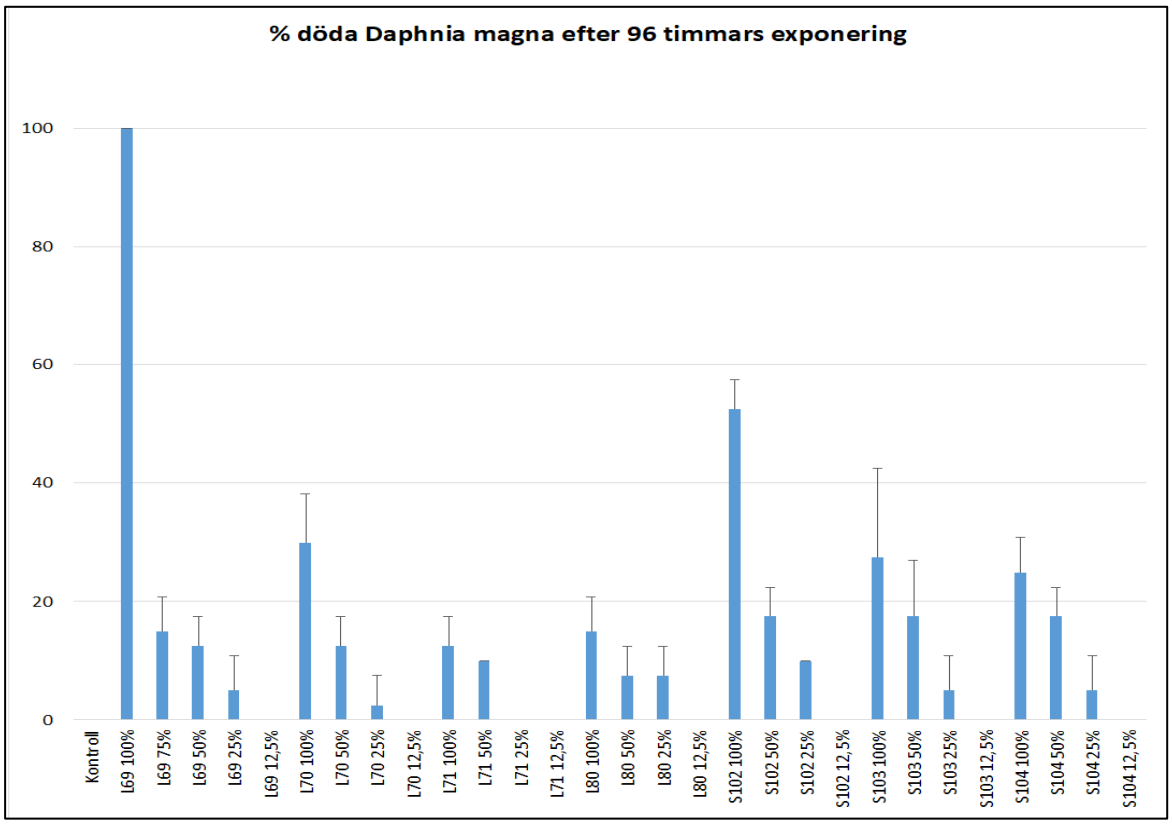


Figure 8: Acute toxicity and immobilisation/mortality in Daphnia magna after 96h exposure in effluents from Lycksele STP and Strömsund STP.

6.8.2 Gene expression analysis in Daphnia magna

Analysis of gene expression using Genotox profile® based on qPCR analysis detected very little guidance for treated samples on causal relationship to certain mechanisms för toxicity, however some biomarkers indicates significant effects in the untreated effluent where 75 % effluent was used in testing due to high acute toxicity, see previous chapter . The results for qPCR analysis are shown in Figure 9.

Compared to unexposed controls (standard Daphnia growth water media), the untreteted effluent (L69) showed significant up-regulation of Cat reflecting ongoing oxidative stress and Gst which indicate increased process activity for converting toxic compunds to less to metabolites or conjugates för excretion (phase 1 and phase 2 reactions). Further Dap1 relating to programmed cell death (apoptosis) was significantly up-regulated. Both MTB and MTC were also slightly up-regulated indicating metal stress. In addition, genes involved in reproductive cycle, Cyp314 and Vasa for Daphnia were up- and down-regulated, respectively.

The effects in treated effluent were much lower as measured in the gene expression analysis where ozonation only exhibit down-regulation of EcRB (gene involved in the molting process). For E-peroxone treated samples (L71 and L80), it is indicated some metal stresss with up-regulated MTA and MTC and in one e-peroxone treated sample (L71) both E74 and USP, both involved in the reproductive cycle are up-regulated and Jhe in L80 was down-regulated which is somewhat inconclusive. In all treated samples, Cat was significantly down-regulated , thus indication that no excess oxidants (O_3, H_2O_2 or radicals formed), remain in the treated effluents.

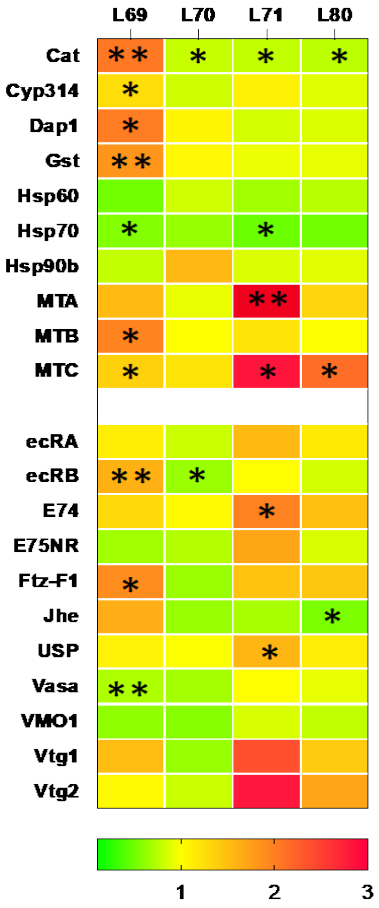
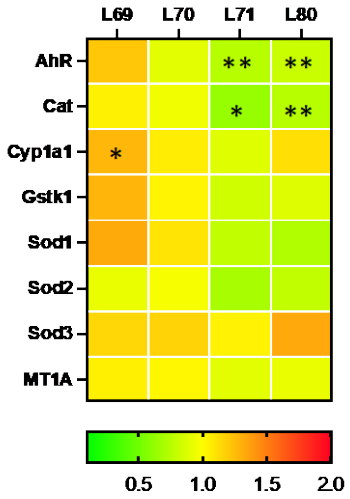


Figure 9: Genotox profile® analysis of gene expression in Daphnia magna post-exposure of water samples from Lycksele STP.

6.8.3 *In vitro* analysis in human cell lines

6.8.3.1 HepG2 cells

The results show that e-peroxone treated water from Lycksele (L71) have a reduced expression of AhR and Cat compared to untreated water (L69), see Figure 10. This indicates that both oxidative stress and exposure to xenobiotics e.g. dioxines or PAHs are reduced after e-peroxone treatment. In the untreated effluent (L69) Cyp1a1 is significantly up-regulated as compared to the control water and suggest ongoing stress from organic xenobitics. This stress reflected by Cyp1a1 cannot be detected in either ozonation- or e-peroxone treated samples.



Figur 10. Genotox profile® analysis of gene expression in HepG2 cells post-exposure of water samples from Lycksele STP.

6.8.3.2 MCF7 cells

MCF7 cell tests aim to detect any estrogenic signaling, but also reflect exposure to organic xenobiotics (e.g., dioxins and PAHs). The results (shown in Figure 11) indicate that Cyp1A1 is affected for L69 and L80 in Lycksele samples. For estrogenic signaling, no significant difference can be detected involvning either Era or Erb, thus no estrogenicity can be attributed to any of the samples. This suggest no presence of estrogenic compounds or to be very low in these effluents. It should be noted that no estrogenic compound (e.g. estradiols) was part of the screening performed for pharmaceuticals in the project.

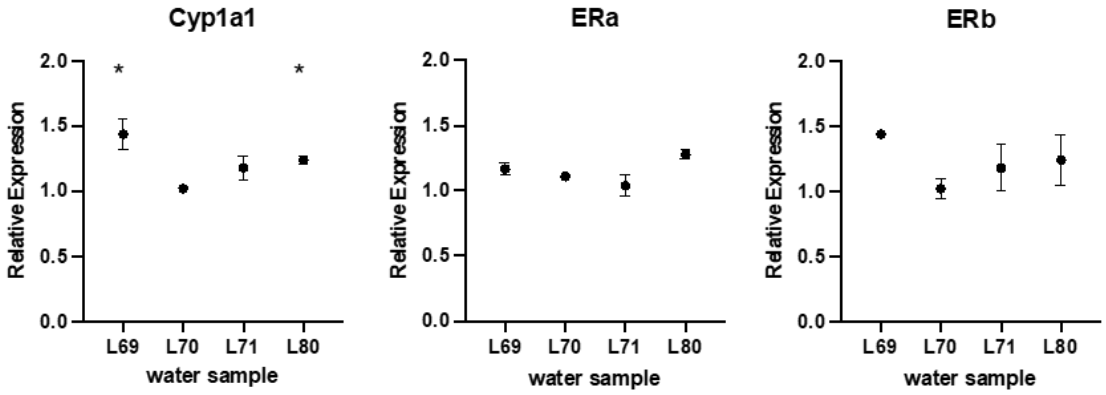


Figure 11. Analysis of gene expression in MCF7 cells after exposure to untreated and treated waters from Lycksele STP.

6.8.3.3 Analysis of immune response in THP1 cells

Exposure of THP1 cells for different water samples without pre-induction using LPS show that IL-6 base level is slightly elevated from e-peroxone treatment (L80). After induction of the immune response with LPS a change can be seen both for the control (LPS) and for untreated water (L69), see Figure 12.

The TNF-a expression levels were not affected in any of the samples from Lycksele with or without LPS induction.

From the results in THP1, cell tests are difficult to intepret any major disturbances regarding immune system functioning in the tested effluents and that indicate presence of immune disturbing substances. Only small differences can be seen compared to control water and also betewen untreted and treated effluents.

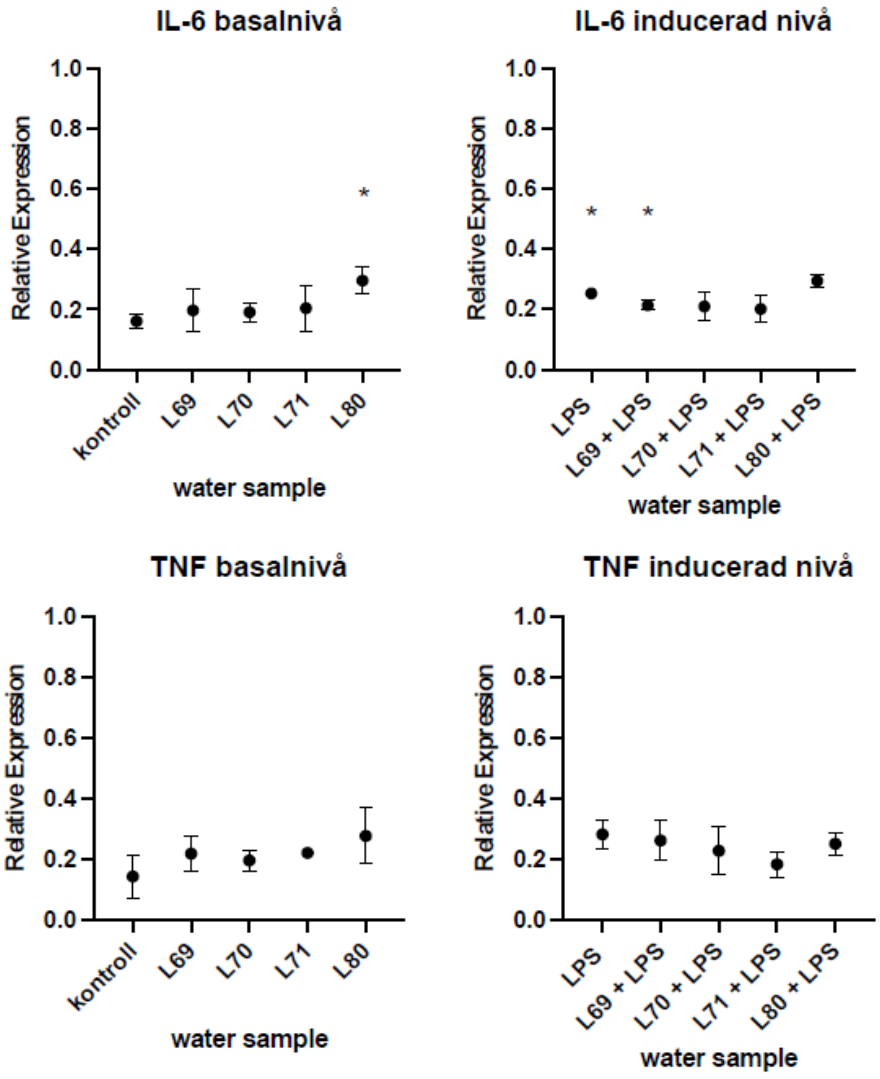


Figure 12. Analysis of protein levels of immune response for biomarkers IL6 and TNF-a in THP1 cells post-exposure to untreated and treated waters from Lycksele STP. Effects on both base levels and LPS-induced levels were tested.

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 hydrogen peroxide during E-peroxone process, the energy consumption is dependent on 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 Lycksele wastewater was estimated to be ~0.318 kWh m^{-3} $year^{-1}$ which is higher than Filip Nilsson 2015 i.e., 0.212 kWh m^{-3} $year^{-1}$. This is because the DOC level is significantly higher in Lycksele wastewater which requires higher ozone dose and ultimately higher production power of the ozone generator. In addition, few components in current pilot have over capacity, thus were consuming more energy than required for the set flows.

On the other hand, the energy consumption requirement for in situ electrochemical generation of hydrogen peroxide, at $[O_3]$:$[H_2O_2]$: 1.2, is estimated to be ~0.065 kWh m^{-3} $year^{-1}$ which is ~20% of the total energy consumption required for ozonation treatment of Lycksele STP effluent. So, the total energy required for E-peroxone process is estimated to be 0.383 kWh m^{-3} $year^{-1}$. It is worth mentioning that recent improvements in the electrochemical cell have resulted in doubled the production of H_2O_2 with almost the 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 ~0.033 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.

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. Lycksele STP is one example which is releasing ~260 kg of pharmaceuticals every year (based on screening of only 98 pharmaceuticals and 2 benzotriazole in this project) into the environment, in this case Umeälven. Considering the 1200 pharmaceuticals sold in the Swedish market and the same detection ratio, the actual pharmaceuticals mass releasing in Umeälven by Lycksele STP 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 for 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 pharmaceuticals in Lycksele secondary wastewater effluent and compared it with conventional ozonation process. Ozonation was able to remove selective pharmaceuticals due to selctive nature of ozone. Many persistent pharmaceuticals 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 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 conditions as only small seasonal variations in results were detected. Slightly lower removal was seen for E-peroxone during the winter testing as compared to other seasons, however it was still significantly higher removal

than ozonation. Overall, E-peroxone process was able to achieve average removal of all pharmaceuticals (detected in Lycksele STP effluent) in the range of 90-95% for different seasons. Many other projects have been studying micropollutants that are mostly ozone reactive. However, many 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 approximately >150 pharmaceuticals to be persistent to ozone oxidation, thus expected to show low or poor removal by ozonation. Removal of these O_3-resistant pharmaceuticals is expected to be improved by E-peroxone process as suggested by the results in this project and other 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. Instead, an overall discussion is made for the implications for both environment and human health regarding emissions of pharmaceuticals if tertiary treatment using AOP would be introduced.

The recipient river Umeälven has an average flow of approx. 228 m^3/s according to SMHI vattenweb[3]. The flow at Lycksele STP for year 2022 was on average approx. 3947m^3/24h (data from LAVAB). Although flows were fluctuating over different seasons, with precipitation intensity and hydropower regulation in the river, an average dilution factor of sewage wastewater effluent of about 6000 times can be calculated. However, this number can vary as the recipient flow varies with hydropower regulation flows which have been estimated to 70 m^3/s at minimum flow and up to 500 m^3/s at maximum flow, thus making the dilution factor in Umeälven to vary between 1750 to 12 500. The authentic dilution will also be affected largely by local conditions such as depth, patterns of main flow directions and overall flow dynamics. Therefore, it is difficult to estimate actual dilution and there will be large temporal variations due to the flow oscillations. Because of that there will always be uncertainties tied to such estimations on which pharmaceuticals will enter into an aquatic system at a certain time and how the fate of pharmaceutical residues will turn out. So, such type of rough estimations should better be used in combination with real measurements of concentrations trends at different locations and time points during the calendar year which has not been the scope of this project.

Out of the many existing and sold pharmaceuticals, only a few can be analyzed in regular laboratories. Besides pharmaceuticals a large number of other contaminants circulates via the sewage. In this study out of 98 screened pharmaceuticals, 45 were detected in Lycksele STP effluent. Further to get an idea of actual situation in term of diversity of micropollutants occurrence at STP, two biocides were also analyzed and interestingly, both of them were detected 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 predicting 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 the 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 endpoint 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.

For this pre-study 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. A substantial and significant difference was shown from the acute tests for *Daphnia magna* where obvious benefits from E-peroxone process were demonstrated. Further, the overall reduction in toxicity was clearly also higher for E-peroxone process in comparison to conventional ozonation.

In vitro tests and gene expressions analysis indicated that untreated effluent from Lycksele STP contained xenobiotics that activated specific biomarkers Cyp1a1 and AhR. These results suggest presence in the effluent of for example dioxine-like compound or other organic contaminants e.g., poly-aromatic compounds, that are known to activate these biomarkers. These effects were not seen for any of the AOP treated samples, thus showing positive treatment effect of both ozonation and E-peroxone. No specific effects were seen for immune response or estrogenic signalling, suggesting the presence of agents causing such events to be low in the Lycksele STP effluent.

The above described battery of bioanalytical tools should not be considered as comprehensive, however, the battery covers many types of effects which are recommended to be studied in this kind of evaluation. and is more comprehensive 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 make 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 supplied H_2O_2. Li et al (2021) estimated the overall cost of electrochemical production of H_2O_2 to be about 0.88 \$/kg H_2O_2 which is economically competitive to the externally supply, i.e., 0.7-1.2 \$/kg H_2O_2 (Ciriminna et al., 2016).

Current pilot system was treating ~215 m^3/day flow of Lycksele effluent 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 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. Lycksele STP, dimensioned for 14000 pe, can 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 for in situ production of H_2O_2 for a year should take into consideration when evaluating the feasibility E-peroxone process for full scale applications.

Testing of E-peroxone process for Lycksele and Strömsund STPs with varying water characteristics and different primary and secondary treatment processes indicates that it can cope under different conditions and the process is less sensitive to changes in the incoming water. However as indicated by some of 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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Umeå 2023-02-24
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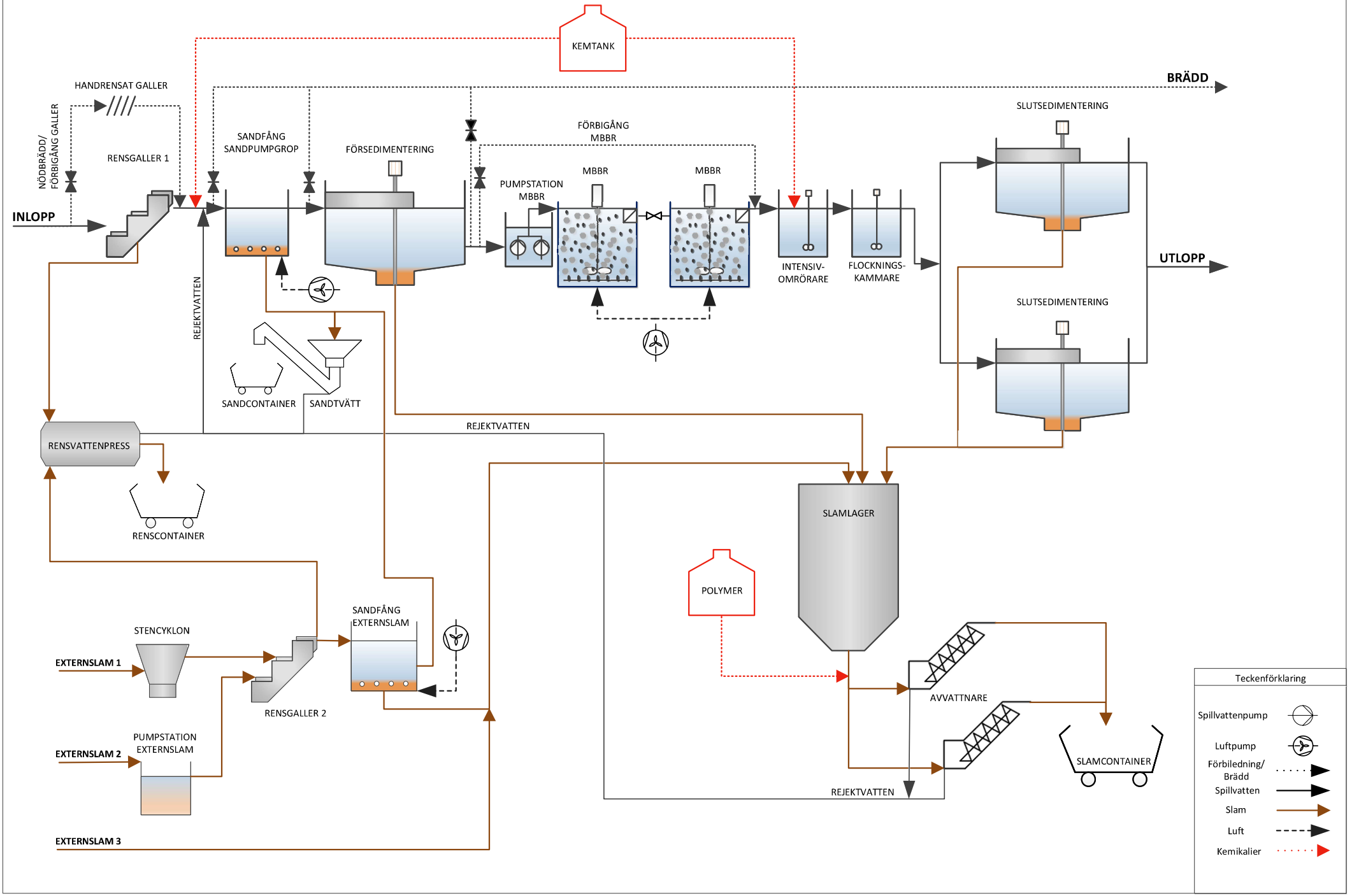
Appendix 1

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

	Winter Testing	**Autumn Testing**	**Summer testing**
Micropollutant	Removal (%) on 14/11/2022	Removal (%) on 12/09/2022	Removal (%) on 04/07/2022
Alfuzosin	100	34	100
Amytriptyline	100	100	100
Atenolol	100	100	100
Atorvastatin	100	100	100
Azithromycin	ND	ND	100
Benzotriazole	80	36	89
Bisoprolol	92	85	87
Bupropion	85	100	100
Caffeine	92	85	100
Carbamazepine	94	100	96
Ceterizine	100	100	100
Citalopram	100	86	100
Clarithromycin	100	100	100
Clindamycin	100	100	100
Codeine	100	100	ND
Desloratadin	ND	ND	2
Diclofenac	100	100	100
Diltiazem	100	100	100
Erythromycin	ND	ND	100
Fexofenadine	100	92	90
Flecainide	91	85	96
Fluconazole	100	88	63
Fluoxetine	ND	ND	100
Irbesartan	92	54	89
Losartan	99	100	100
Memantine	78	70	84
Methyl-benzotriazole	100	100	85
Metoprolol	100	71	95
Metronidazole	81	ND	ND
Mirtazapine	100	100	100
Oxazepam	64	90	100

Paracetamol	94	98	100
Propranolol	ND	100	100
Rosuvastatin	100	88	100
Sertraline	100	100	100
Sulfamethoxazol	100	100	ND
Tramadol	ND	94	95
Trimethoprim	100	100	63
Venlafaxine	100	100	100
Total No. Of micropollutants detected	***33***	***34***	***36***
Average removal	***95***	***90***	***93***


KEMTANK
BRÄDD
HANDRENSAT GALLER
NÖDBRÄDD/
FÖRBIGÅNG GALLER
RENSGALLER 1
INLOPP
SANDFÅNG
SANDPUMPGROP
FÖRSEDIMENTERING
FÖRBIGÅNG
MBBR
PUMPSTATION
MBBR
MBBR
MBBR
INTENSIV-
OMRÖRARE
FLOCKNINGS-
KAMMARE
SLUTSEDIMENTERING
SLUTSEDIMENTERING
UTLOPP
REJEKTVATTEN
SANDCONTAINER
SANDTVÄTT
REJEKTVATTEN
RENSVATTENPRESS
RENSCONTAINER
SLAMLAGER
POLYMER
STENCYKLON
EXTERNSLAM 1
SANDFÅNG
EXTERNSLAM
RENSGALLER 2
PUMPSTATION
EXTERNSLAM
EXTERNSLAM 2
EXTERNSLAM 3
AVVATTNARE
SLAMCONTAINER
REJEKTVATTEN
Teckenförklaring
Spillvattenpump
Luftpump
Förbiledning/
Brädd
Spillvatten
Slam
Luft
Kemikalier

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

RAPPORT BioImpakt AB Örebro 20221229

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Innehållsförteckning

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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 Lycksele 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 visas i tabell 1.

Tabell 1. Koncentrationer av läkemedelsubstanser I testade vattenprover.

Substans	Site	Lycksele ARV			
	Prov ID	L69	L70	L71	L80
	LOQ	Obehandlat vatten	Ozonering	E-peroxone	E-peroxone
	ng/L	ng/L	ng/L	ng/L	ng/L
Alfuzosin	4	77	<LOQ	51	
Atorvastatin	10	216	<LOQ	<LOQ	
Bisoprolol	3	290	10	44	
Clarithromycine	3	223	<LOQ	<LOQ	
Clindamycine	3	16	<LOQ	<LOQ	
Codeine	15	995	<LOQ	<LOQ	
Diclofenac	10	1946	701	<LOQ	<LOQ
Diltiazem	1,5	61	<LOQ	<LOQ	
Fexofenadine	10	195	<LOQ	15	
Flecainide	1,5	230	<LOQ	36	
Paracetamol	30	16286	532	284	561
Rosuvastatin	20	2087	<LOQ	257	
Sulfamethoxazol	15	109	<LOQ	<LOQ	
Tramadol	15	1059	<LOQ	67	
Trimethoprim	3	143	<LOQ	<LOQ	<LOQ
Venlafaxine	20	1204	<LOQ	<LOQ	
Propranolol	20	372	<LOQ	<LOQ	
Amytriptyline	10	203	<LOQ	<LOQ	
Atenolol	15	1105	39	<LOQ	
Bupropion	3	137	7	<LOQ	
Carbamazepin	7,5	293	13	<LOQ	24
Citalopram	15	273	16	38	17
Metoprolol	15	3503	133	1004	184
Mirtazapine	15	109	<LOQ	<LOQ	
Caffeine	20	195398	4059	28830	
Ceterizine	15	285	<LOQ	<LOQ	
Losartan	10	939	<LOQ	<LOQ	
BZ	50	565	227	363	135
MBZ	50	956	<LOQ	<LOQ	70
Desloratidin		*15*			
Fluconazole	7,5	86	72	11	60
Irbesartan	3	62	50	29	10
Memantine	3	97	34	29	16
Oxazepam	10	218	32	22	30
Sertraline	10	15	<LOQ	<LOQ	

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.

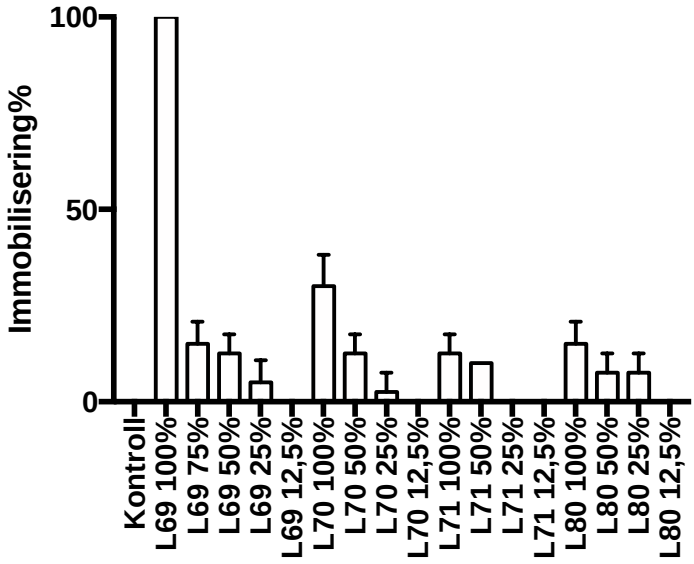
Vattenloppa är vanligtvis bland de känsligaste organismerna i en s.k. artkänslighetsfördelning där olika arters effektmått för ett visst medium eller agens jämförs. Uppnås ingen effekt på vattenloppa är det mindre sannolikt att samma testkoncentration ska ge upphov till negativa effekter på andra organismer.

RESULTAT
Resultat från utförda biologiska tester för obehandlat vatten, som enbart genomgått rening via konventionella reningsteg 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 Lycksele (L) uppmättes ett IC_{50} värde motsvarande 87,5% för L69.För proverna L70, L71 och L80 kunde inget LC_{50} värden uppmätas då effekten var låg.

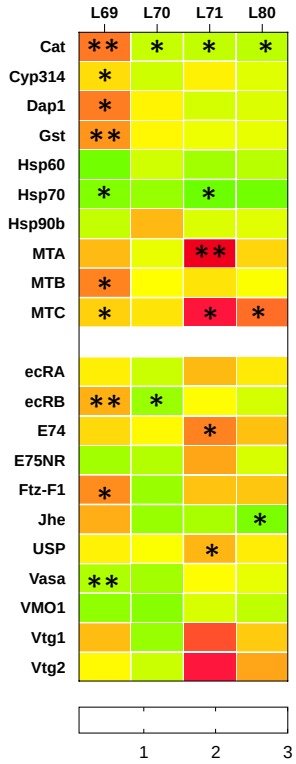
Utifrån akuttoxiciteten kunde LC_5 värden uppskattas. För vatten från Lycksele uppskattades LC_5 till ca 25% vatten för L69, ca 32% för L70, ca 38% för L71 och ca 18% för L80.

Från dessa resultat användes 100% vatten för samtliga genotox profile® analyser på *Daphnia magna*, förutom för vatten L69 där mortaliteten var för hög vid 100% vatten och 24 timmars exponering. Följaktligen användes 75% vatten från L69 för analyserna.

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.

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.



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

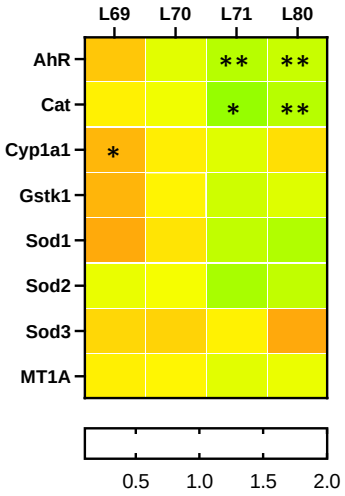
Analys av lakvattnets effekt på genuttryck jämfört med ”Daphnia standard water”, kontrollvatten, visar att effekterna på *Daphnia magna är* störst med det obehandlade vattnet från Lycksele (L69). Det vattnet kunde endast användas i 75% av ursprungskoncentration eftersom ospätt vatten (100%) resulterade i mortalitet efter 24 timmar. Effekterna jämfört med kontrollvattnet var minst efter ozonering (L70) med endast EcRβ uppreglerat. Efter e-peroxone behandling (L71 och L80) syntes ökat genuttryck av MTA, MTC, och E74 jämfört med kontrollvattnet.

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), 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

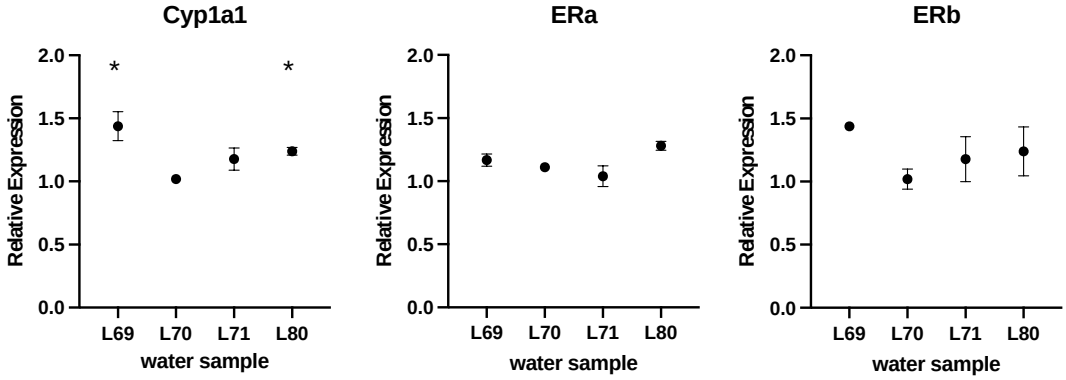
HepG2 celler har sitt ursprung i leverceller och är därför väl lämpade för att testa toxicitet. Cellerna exponerades för de olika vattenproverna utan spädning. Exponeringen fortgick i 24 timmar varefter cellerna användes för extraktion av RNA.



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

Resultaten visar att vatten från Lycksele som behandlats med e-peroxone har ett minskat uttryck av AhR och Cat jämfört med obehandlat vatten vilket indikerar att både oxidativ stress och exponering för dioxin/PAH-lika ämnen minskats efter e-peroxone behandlingen. I obehandlat vatten (L69) påvisats ökat genuttryck av Cyp1A1 vilket indikerar att organiska ämnen som stimulerar uttrycket av Cyp1A1 förekommer i obehandlat vatten, en effekt som försvinner efter behandling med både ozon och e-peroxone, 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.



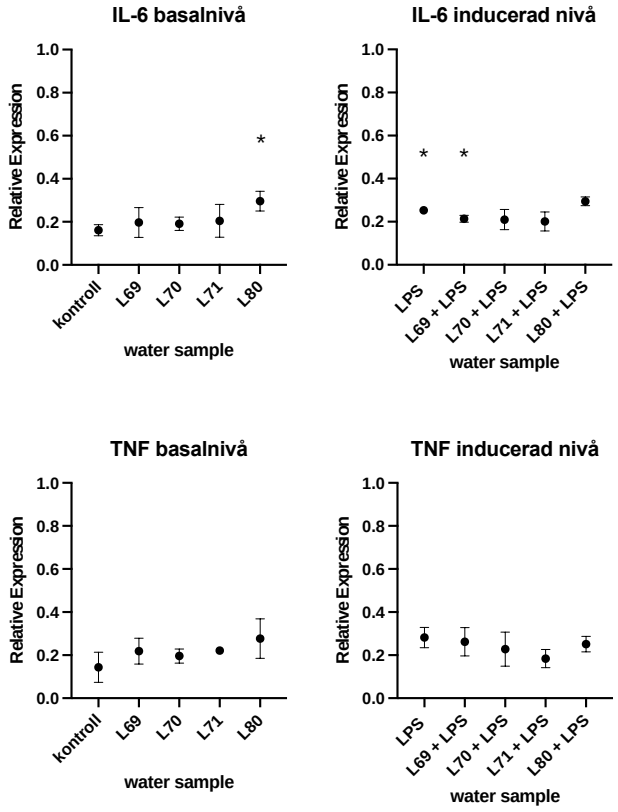
Figur 4. Analys av genuttryck hos MCF7 celler efter exponering för vatten från Lycksele reningsverk.

Resultaten visar att Cyp1a1 är påverkat i både L69 och L80 även om effekten är minimal. För östrogen signalering kan ingen statistiskt signifikant förändring iakttas hos något av proven vilket indikerar låg närvaro av östrogena ämnen i samtliga prover inkl. det obehandlade provet L69. Ingen kemisk analys har utförts för östrogena ämnen (inkl 17-β estradiol) vid screening av läkemedelssubstanser i vattenprover.

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 vattenprov L80. Efter inducering av immunförsvaret med LPS kan man se en förändring i kontrollen (LPS) samt i obehandlat vatten (L69).

TNF-α nivåerna påverkades inte i vattenproverna från Lycksele, varken på basalnivå eller efter LPS-stimulering.



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

Utvärdering av resultat och slutsatser

Utförda akuttoxicitetstester med *Daphnia magna* visar att mycket hög toxicitet kan påvisas i avloppsvatten L69 som enbart genomgått konventionella reningssteg. Genom behandling med e-peroxone reduceras akut toxicitet med ca 85 % och med ozonering är reduktionen ca 70 %. Resultaten är i paritet med graden av reduktion av läkemedelssubstanser efter behandling.

Bland specifika mekanismer som analyserats kan sammanfattningsvis sägas att låg/ingen effekt på immunsystemet uppvisas på samtliga prover. Tester med MCF7 celler indikerar vidare att exponering för östrogena ämnen är låg i samtliga prover.

Vidare uppvisade tester i både HepG2 celler och MCF7 celler att Cyp1A1 är uppreglerad för obehandlat prov L69. Uppreglering i Cyp 1A1 för L69 avspeglar att det förekommer exponering för vissa organiska ämnen som kan inducera uppregleringen, sådana ämnen kan vara dioxinlika och PAH-lika ämnen. Denna effekt försvinner efter behandling med både ozon och e-peroxone,

För HepG2 celler visar testerna även att e-peroxone behandling minskat ett minskat uttryck av AhR och Cat jämfört med obehandlat vatten L69 vilket indikerar att både oxidativ stress och exponering för dioxin/PAH-lika ämnen minskats efter e-peroxone behandlingen.

Det kvarstår effekter som kan påvisats framför allt vad gäller markörer som indikerar ökad metallexponering (gener som indikerar oxidativ stress och uttryck av metallothionein) vilket kan vara en reflektion av att organiskt material brutits ned under oxidationsbehandling och därmed kan frisätta och åtminstone temporärt öka graden av metallexponering vilket testerna indikerar. Med tanke på de kraftigt minskade nivåerna av läkemedel efter behandling med e-peroxone så kan effekterna även bero av andra ingående ämnen som inte ingått i den kemiska analysen av testade vatten.

Örebro 20221228

För BioImpakt AB

Per-Erik Olsson
Projektledare

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

Appendix 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 Lycksele Avfall & Vatten AB**	**Kommentarer Envix**
<u>Tidplan för utförande av projekt:</u> Augusti 2021 till oktober 2022.	Nej, förseningar p.g.a. både pandemi- och krigsrelaterade faktorer		Nytt system byggt och vissa 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 578 869 kr. Projekt har äskat mer pengar i augusti 2022. NV gav ej bifall till mer pengar. Se vidare specifikation under ekonomisk uppföljning nedan.	Pengar äskades tidigt i augusti till projektet pga aviserade kostnadsökningar. Negativt svar från NV kom sent i projektets slutskede	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 som krävdes för komplett vintersäkring då anläggningen stod utomhus.

Måluppfyllning utifrån syftet med förstudien			
Huvudsyftet: Att bedöma dagens rening med avseende på mikroföroreningar och utvärdera en ny avancerad 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.	Från Lavabs del har det krävts att tekniker funnits på plats under testning och provtagning för ev. åtgärder under projektets gång	Utvärdering beskrivs ingående i huvudrapporten, men reningsgrad som kunde uppvisas med ny avancerad oxidationsprocess uppgår till > 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. Ett relä överhettades och byttes under sommartestning, men efter byte gick anläggningen som planerat. Viss frysproblematik i utgående slangar har hanterats och åtgärdats under tester i vinterförhållanden. Lavabs driftspersonal har varit till stor hjälp för att lösa praktiska frågor under drift och genomförande samt viss vattenprovtagning.
Analysomfattning:			
<u>Kemisk analys:</u> Större screening av läkemedel ca 100 st och selektion av relevanta ämnen som behövs för att studera effektivitet av	Ja		Totalt har läkemedelsanalys utförts för ca 130 prover i projektet. Ytterligare ett fåtal i förtester och under optimering samt baskarakteristik

reningsmetod. I övrigt baskarakteristik för avloppsvatten.			
<u>Biologisk analys:</u> Både akuta tester och relevanta effektbaserade in vitro tester för relevanta markörer och tillräcklig känslighet och specificitet bör tillämpas i projektet	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.
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		En svensk sammanfattning ingår även i slutrapporten vars huvudrapport är på engelska
- Löpande kommunicera runt projekt på LAVABs och Lycksele kommuns hemsida	Ja	Pressrelease initialt, löpande uppdateringar har gjorts	Envix har försökt bistå LAVAB med nödvändig information för deras kommunikationsarbete.
- Arrangera ett större regionalt seminarium om projektet för branschaktörer	Nej, följer isåfall efter projektavslut.		Envix är villiga att ha en dragning om projektet vid sådant arrangemang
Kommunikation i övrigt			

Projektmöten	Ja	Genomförts med tydliga intervaller och med god återkoppling av genomförandet och testresultat	Avstämningsmöten har hållits 1 gång i månaden där pågående och planerade aktiviteter delgivits information kring. 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.
Övrigt	Ja, seminarium i samband med styrelsemöte hos LAVAB	En bra och tydlig genomgång av projektet och redovisning av de resultat som då fanns tillgängliga	Envix en genomgång om projektet och framkomna resultat 9 december för LAVABs styrelse.
Mediakontakter	Ja, kommunens pressrelease och kommunikation via hemsidan om projektet.	Lavab har inte fått frågor från externa	Envix har svarat på tekniska frågor som ställts vid kommunikation som gått via Lycksele kommuns hemsidor.
Sekretess		Tydlig och har efterlevts	Ett sekretessavtal skrevs med LAVAB med anledning av pågående ansökningsprocess runt immateriella rättigheter för nya avancerad oxidations -process. Detta har fungerat mycket bra och LAVAB har respekterat 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		Envix föreslog en betalplan inför projektet med fasta tidpunkter för reglering utefter som kostnader uppstod. Betalplanen hade merparten av kostnader tidigt i planen som avsåg för byggnation av pilotanläggningen vilket uppstod tidigt i projektet. LAVAB 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 578 689 sek.	Redovisat vid äskande av mer medel för projektet till NV	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 gjordes till LAVAB 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 drabbade Envix. Redovisning av merkostnader har redan skett vid separat framställan.
Redovisning av kostnadsposter i projekt	Ja	Enligt specifikationer nedan. Utdrag från LAVABs ekonomisystem.	

ENVIX
WWW.ENVIX.SE

LAVAB
Lycksele Avfall & Vatten AB

Utdrag LAVAB ekonomisystem exkl. förväntad kostnad om 505 209 sek ang. slutreglering enligt uppgjord betalplan mellan LAVAB och Envix.

T	VT	Ver.nr	#	Ver.datum	Period	Konto	Konto (T)	Ansvar	Projekt	Projekt (T)	Verksamhet	Motpart	MK	Text	Belopp
B	ED	23018487	1	2022-01-03	202112	561110	Diesel	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0		838,42
B	ED	23021866	1	2022-10-04	202210	655200	Elkonsulter	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0	br vikströms	1 244,89
B	ED	23020119	1	2022-05-11	202206	541040	Elmaterial	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0		4 116,79
B	ED	23020120	1	2022-05-11	202206	541040	Elmaterial	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0		29,86
B	ED	23022554	1	2022-11-25	202212	541040	Elmaterial	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0		2 744,61
B	ED	23022619	2	2022-12-01	202212	546000	Förbrukningsmatr inkl småverktyg	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0	rördelar	1 958,42
B	HB	10001111	1	2022-06-28	202206	541010	Rördelar/slang/brunnar/VVS	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0	korr 23020380 ej invest	347,23
B	ED	23018883	2	2022-01-31	202202	655900	Övriga konsulter	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0	Envix	382 123,00
B	HB	10000974	1	2021-11-30	202111	655900	Övriga konsulter	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0	korr ver 23017736 - fel konto	80 663,00
B	ED	23017400	1	2021-09-30	202110	655900	Övriga konsulter	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0	Envix Nord	1 231 745,00
B	HB	10000936	1	2021-09-30	202109	655900	Övriga konsulter	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0	Korr ver 23017111 - fel konto	1 189 663,00
B	ED	23018634	1	2021-12-31	202112	655900	Övriga konsulter	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0	Envix	165 759,00
B	ED	23018137	1	2021-11-30	202112	655900	Övriga konsulter	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0	Envix	278 505,00
B	ED	23019621	2	2022-03-31	202204	655900	Övriga konsulter	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0	Envix se bilaga	68 171,00
B	ED	23020918	2	2022-06-30	202207	655900	Övriga konsulter	611000	576221	Pilotproj rening av läkemedel - NV-21-006420	76310	800	0	envix	54 918,00
															3 462 827,22