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Occurrence patterns and ecotoxicity of organic micropollutants in Israeli seasonal pools

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מילות מפתח

Runoff, chemical pollution, aquatic risk, winter ponds, mixtures, science-based management.

תום טופז

תקציר

בריכות עונתיות הן גופי מים ארעיים המתמלאים בחורף ומתייבשים ברובם או במלואם בעונה היבשה. בריכות אלו מהוות פרוזדורים אקולוגיים מימיים ותומכות במגוון ביולוגי גדול עם אוכלוסיות מגוונות של חסרי חוליות, חרקים, דו-חיים וצמחייה. המינים המתפתחים בבריכות עונתיות מאופיינים במחזורי חיים קצרים יחסית ונדרשים להשלים את מהלך חייהם בהידרופריודה (כאשר הבריכה מלאה במים) קצובה בזמן. קיומן ושגשוגן של בריכות עונתיות מצוי בסכנה מתמדת אשר גוברת במקביל לפיתוח האנושי כתוצאה משימושי קרקע אינטנסיביים, וקיים פער ידע משמעותי על השפעות כימיקלים המוסעים אל הבריכות עם הנגר (ובנתיבים נוספים) על אוכלוסיית הבריכות. מטרת מחקר זה הייתה להציג באופן שיטתי את נוכחותם של מיקרו-מזהמים, כגון תרופות ומוצרי טיפוח אישי, כימיקלים להגנת הצומח, וכימיקלים תעשייתיים, במרכיבי הבריכות השונים (סדימנט, מים וצמחיה) ולאורך עונות השנה (סתיו, חורף ואביב). על בסיס הממצאים בוצעה הערכת סיכון שמזהמים אלה מציבים לתפקוד המערכת האקולוגית.

במהלך המחקר נמצאו סה״כ 176 מזהמים שונים במים, בסדימנטים, ובצומח מ-12 בריכות עונתיות הפזורות לאורכה של ישראל. נמצאה קורלציה חלשה בין שימושי קרקע עירוניים וחקלאיים לבין רמת הזיהום כך שמזהמים נמצאו גם בבריכות המנקזות אזורים עם פעילות אנושית נמוכה. מספר המזהמים בבריכות וריכוזם ירדו במשך ההידרופריודה, דינמיקה עונתית זו נובעת ככל הנראה מתהליכי פירוק או ספיחה של המזהמים. הערכה של תהליכי ספיחה בוצעה על ידי בחינת החלוקה של מזהמים בין סדימנטים, המים והצמחייה ונמצאה עדות לספיחה או קליטה פוטנציאלית של תרופות על ידי צמחיית המים. הערכה חישובית של פוטנציאל הרעילות של תערובת המיקרו-מזהמים לאצות ולסרטנאים חשפה רמות סיכון משתנות בין בריכות שונות ועונות השנה, כאשר חלק מהבריכות מציגות רמת סיכון (Risk Quotient) מעל לסף המקובל RQ<1. עבור אצות ב- 55% מהבריכות בחורף חושב סיכון מעל ערך הסף עם ערכי RQ של 118-9084% מערך הסף ובאביב ב- 45% מהבריכות חושבו ערכים של 119-2134% מערך הסף. לעומת זאת, עבור סרטנאים, רק ב- 18% מהבריכות בחורף חושב RQ גבוה מ-1 עם ערכים של 138-396% מערך הסף ובאביב בבריכה אחת (9%) חושב סיכון גבוה מערך הסף (106%).

תוצאות המחקר מדגישות את היקף הזיהום הסביבתי על ידי מיקרו-מזהמים אורגניים, גם באזורים מרוחקים מפעילות אנושית ישירה, ומצביעות על השפעת השימוש האנתרופוגני בקרקע על איכות המערכות האקולוגיות בבריכות. עם זאת, מגוון מאפייני הבריכות והשונות בין אגני הניקוז מציבים אתגר לקבלת הבנה מרחבית אחודה ונדרשת התייחסות נקודתית לכל בריכה ומאפייניה (כפי שנעשה ומפורט בדו״ח). במרבית הבריכות תוצאות הערכת הסיכונים וההתאמה למדדי עושר מינים היו גבוליות כך שמצד אחד לא ניתן לשלול השפעה כימית על מאכלסי הבריכה, אך מצד שני בפעולות אופרטיביות מינימליות ניתן יהיה לכאורה לצמצם משמעותית את חשיפת הבריכות למיקרומזהמים המסכנים אותה. המלצות להפחתת זיהום וניהול סיכונים בבריכות עונתיות כוללות יישום אזורי חיץ, אמצעים לצמצום זליגה או רסס של מוצרים להגנת הצומח, טיוב הנגר טרם כניסתו לבריכה, חיזוק צמחייה מימית לקליטת תרופות, וניטור ואכיפה מוגברים וממוקדים באזורים פגיעים. דו״ח מחקר זה מציג לראשונה פריסה מרחבית של תערובות מזהמים מורכבות בבריכות חורף ונותן בסיס והמלצות להמשך ניטור וניהול בריכות חורף לצמצום סיכונים של זיהום על ידי כימיקלים לצורך תמיכה בתפקודן המאוזן והבריא של הבריכות ובשגשוג המגוון הביולוגי המאכלס אותן.

Abstract

Seasonal pools are temporary water bodies that fill during the rainy season and dry out mostly or completely during the dry season. These pools form aquatic ecological corridors and support great biodiversity with diverse populations of invertebrates, insects, amphibians, and vegetation. The species that develop in seasonal pools are characterized by relatively short life cycles and are required to complete their life course during the limited hydroperiod when the pool is full of water. The existence and prosperity of seasonal pools is in constant danger, which increases in parallel to human development as a result of intensive land uses, and there is a significant knowledge gap about the effects of chemicals carried with the runoff on the population of the pools. This study systematically presents the presence of micropollutants, such as pharmaceuticals and personal care products, plant protection chemicals, and industrial chemicals, in the various pools' matrixes (sediment, water and vegetation) and throughout the seasons (Autumn, Winter, and Spring), and evaluates their ecological risks. A total of 176 different pollutants were found in water, sediments, and vegetation samples from 12 seasonal pools spread along Israel. A weak correlation was found between urban and agricultural land use and the variety and concentration of pollutants decreased during the hydroperiod, showing seasonal dynamics related to decomposition or adsorption processes of the pollutants. An assessment of adsorption processes was performed by examining the partitioning of pollutants between sediments, water, and vegetation and evidence of potential adsorption or absorption of pharmaceuticals by vegetation was detected. A computational assessment of the micropollutants mixture toxicity potential to algae and crustaceans revealed variable risk levels between pools and seasons, with some pools presenting risk quotients above the acceptable threshold of RQ<1. For algae, 55% of the pools in winter with RQ values ranging from 118% to 9,084% of the threshold value. In spring, 45% of the pools presented unacceptable risk quotients of 119-2,134% above the threshold. Crustaceans was under unacceptable risk in only 18% of the pools during winter with RQ values of 138-396% above the threshold and in spring one pool had unacceptable risk of 106% above the RQ threshold. The results of the study emphasize the extent of environmental pollution by organic micropollutants, even in areas remote from direct human activity, and indicate the effect of anthropogenic land use on the quality of the ecosystems in the pools. The variety of characteristics of the pools and the differences between their watersheds pose a challenge to obtain a unified spatial understanding and methodology and a pool-specific approach is required to promote pollutant mitigation in pools subjected to risk (as was done and detailed in the report). In most of the pools the risk was limited so that on the one hand, an impact on the pool's inhabitants cannot be ruled out, yet on the other hand, with minimal operative actions it will be possible to mitigate micropollutants and significantly reduce the risk to the pools. Recommendations for reducing pollution and risk management in seasonal pools include the implementation of absorbing patches, measures to reduce spillage or spraying of plant protection products, runoff treatment prior to its entry into the pool, enforcing pool's vegetation for pharmaceuticals removal, and targeted monitoring and enforcement in vulnerable areas.

Contents

1. Introduction

Seasonal pools, also known as ephemeral wetlands or winter pools, are temporary water bodies that fill during the rainy season and dry out mostly or completely during the dry season (Zedler, 2003; Blaustein and Schwartz, 2001). These pools, ranging from small to large areas, are prevalent globally, especially in regions with a Mediterranean climate (Gafny, 2013). According to estimates, there were about 1,500 seasonal pools in the Mediterranean area of Israel, some of them covered large areas and others functioned as irrigation pools around villages and agricultural areas. Most of these pools disappeared due to human development and many of the pools that remain today are located near population centers, and are accessible as nature sites, recreational activities, and infrastructure for promoting research and education activities for the protection of biological diversity (Elron et al., 2021).

The hydrology of seasonal pools, influenced by draining precipitation and groundwater, determines their duration of standing water, impacting biodiversity and ecological processes (Zedler, 2003). These pools support diverse populations of invertebrates, insects, amphibians, and vegetation, playing a crucial role in species conservation (Fahd et al., 2009; Picazo et al., 2012; Jakob, 2003). Their short hydroperiod and relative isolation create refuges for rare and endangered species, contributing to their ecological significance (Zedler, 2003; Elron and Gafny, 2011).

However, changes in land use, drainage practices, and extreme weather events, threaten the existence of seasonal pools (King, 1998; Garmendia and Pedrola-Monfort, 2010). Pollution from urban and agricultural runoff deteriorates water quality and may impact habitat conditions and biodiversity (Battaglin et al., 2009; Topaz et al., 2020). Organic micropollutants, including pharmaceuticals and pesticides, enter these pools through various pathways, posing risks to aquatic organisms (Naidu and Wong, 2013; Samadi et al., 2022).

Understanding the fate and transport of organic micropollutants in seasonal pools is essential for assessing their ecological impacts and establishing science-based tools for rehabilitating and managing seasonal pool ecosystems. (Mina et al., 2018). Advanced analytical techniques have improved our ability to detect and quantify these pollutants, facilitating ecotoxicity evaluations and risk assessments (Noguera-Oviedo and Aga, 2016). Risk assessment considers both the hazards posed by pollutants and the exposure levels, recognizing the potential synergistic effects of multiple compounds (Escher et al., 2021).

In Israel, comprehensive information on organic micropollutants in seasonal pools is absent, highlighting the need for research to evaluate their occurrence, patterns, and potential ecotoxicity effects. By identifying and quantifying these pollutants and assessing their toxicity to pool inhabitants, this study aims to provide scientifically grounded recommendations for the management and conservation of seasonal pools in Israel and beyond.

2. Methods and methodology

For this study, 12 pools were selected across Israel, differing by size, mode of formation, geographical area, basin characteristics, maintenance, etc. (figure 1). All pools were sampled for sediments, water and plant tissue, with the exception of "Beit Keshet" Pool, which didn't have a significant hydroperiod, possibly due to human-interference in the contributing watershed (Table 1). The pools were divided into geographic areas: north, center and south and the sizes of the pool area ranged from 79 to 42,542 square meters (Table 1). Defining the area that affects the quality of the pools' water, by different land uses, was done using two conceptual approaches: (1) examination of the drainage basin of each pool, and (2) examination of the potential effect of a radius of 1 km from the pools' edges (figure 1). The latter considers that some chemicals (e.g. plant protection products) may reach the pools via emissions or spray drifts transporting through the air compounds like. Characterization of the area and size of the pools and basins as well as the maps production was done using QGIS and layers originating from the Jewish National Fund - Keren Kayemeth LeIsrael (JNF-KKL, 2023) and the Israel government's GIS data (https://www.govmap.gov.il, accessed in 2023).

Table 1: Selected seasonal pools characterization and samples type collected for water quality and micropollutants analysis.

Location	District	Cor "N	Cor "E	Pool Area	Samples Collected			
				m^2	Sediments	Winter water	Spring water	vegetation
El Mo'ayer	North	33.081894	35.729339	1,708	V	V	V	V
Naphach	North	33.065111	35.740611	348	V	V	V	V
Katzabia	North	32.975744	35.717553	1,236	V	V	V	V
Bajuria	North	32.863779	35.789129	4,232	V	V	V	V
Beit Keshet	North	32.720997	35.406829	8,897	V	-	-	-
Horshan 2	Centre	32.580662	35.013849	79	V	V	V	V
Horshan 3	Centre	32.578756	35.017684	687	V	V	V	V
Hodaya	South	31.682032	34.65525	702	V	V	V	V
Eitan	South	31.566761	34.73742	900	V	V	V	V
Horshim	Centre	32.141778	34.969161	225	V	V	V	V
Batich	Centre	32.464481	34.938606	7,400	V	V	V	-
Hadera forest	Centre	32.420156	34.890219	42,542	V	V	V	V

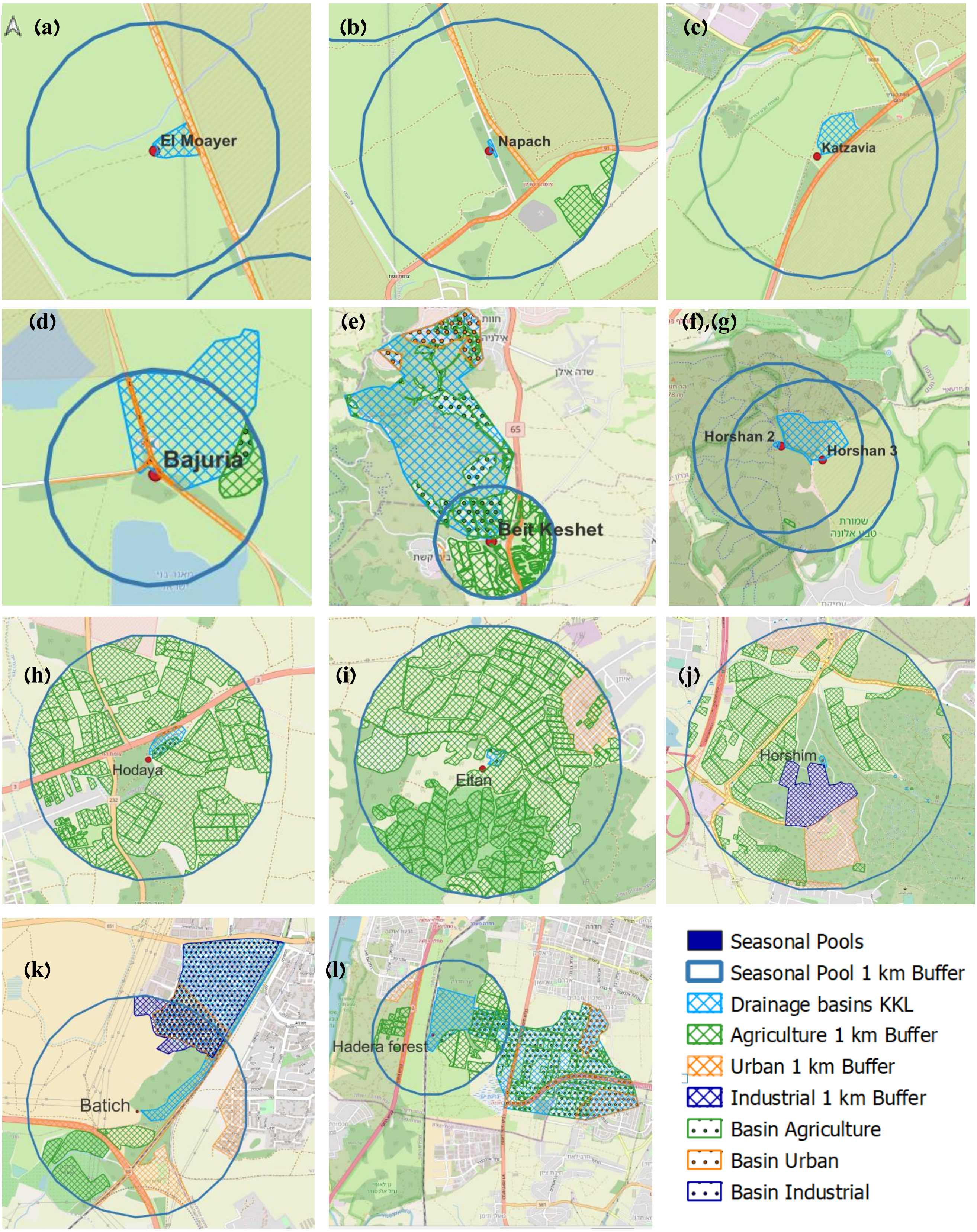


Fig 1. Seasonal pools' watersheds and contributing 1 kilometer radius. : (a) El Mo'ayer , (b) Naphach, (c) Katzabia, (d) Bajuria. (e) Beit Keshet, (f) Horshan 2, (g) Horshan 3, (h) Hodaya, (g) Eitan, (h) Horshim, (i) Batich and (j) Yaar Hadera. Maps were created by qGIS using layer from "DATA GOV", Government databases.

2.1 Sampling

Sediments: Sediment samples were taken once during the dry season from 2 to 4 sampling points of the top layer (1-2 cm) of the pool's sediment. The collected samples were batched in one glass jar to obtain a representative composite sample for each of the pools. In the pools that were not completely dry, samples were taken from three dry locations in the margins of the area that was expected to be flooded with hydroperiod. Sediment samples were lyophilized and immediately stored in a freezer (-20°C). The sediments samples were tested for organic micropollutants, metals, content of carbon and nitrogen and grain size distribution.

Water: Water sampling at all sites was performed twice, Once, at the beginning of the rainy period shortly after the pools fill up, and again, towards the middle or end of the hydroperiod. Each water sample was collected from three different points at each pool, from the middle of the water column into clean glass vessels. In preparation for analysis organic pollutants the sampled water was filtered using 90 mm GFF filters. After that, frozen immediately and kept in the freezer (−20 C°) until further treatment. The water samples were tested for organic micropollutants, metals and nutrients.

Vegetation: Vegetal material was collected from the pools themselves into clean vessels, freeze-dried, and kept in the freezer (−20 C°) and later lyophilized and organic micropollutants screening.

2.2 Organic micropollutants Analyses

All samples (water, sediments and plant tissue) were analysed for ~ 2,500 organic micropollutants and metabolites using the high-resolution LC-MS / MS method developed in the National and Kapodistrian University of Athens, department of chemistry (Aalizadeh et al. ,2021; Thomaidis et al., 2022). For calculation purposes, pollutants detected below LOQ (Limit of quantification) were reported according to the LOD (Limit of detection) value of each pollutant, and for pollutants found to be lower than the LOD, a concentration of zero was reported.

2.3 Ecotoxicity assessment

Toxicity assessment for algae (EC50) and crustaceans (LC50) was calculated for each individual compound using available experimental data collected from reliable databases: ECOTOX (ECOTOX Project, 2022) as first priority or NORMAN (NORMAN Network, 2022) as second priority databases (Figure 2). When these options weren't available, an evaluation was performed using the Ecological Structure Activity Relationships (ECOSAR). Division factors were utilized to evaluate the Predicted No-Effect Concentration (PNEC) for the aquatic habitat. The chronic test assessment factor signifies the shift from controlled laboratory conditions to real-world field conditions, incorporating an additional factor for converting the acute test results to chronic levels (European Chemicals Bureau, 2003). The risk quotients (RQ) were calculated for each monitored compound (equation 1). Risk quotient (RQ) ≥ 1 was considered an unacceptable risk for the aquatic habitat. Risk quotient 1≥ (RQ) ≥ 0.1 was considered moderate risk.

Equation 1. Risk quotient RQ Calculation; C_i- individual concentration; PNECi- predicted non effect concentration.

$$RQ = \frac{C_i}{PNECi}$$

RQ values computed for the mixture of the investigated compounds in each sample. Hence, we initially estimated the Predicted No-Effect Concentrations (PNECs) for each compound within each taxonomic group (algae or Crustaceans) using the prioritization and assessment factor outlined below. PNEC values were determined for most compounds, specifically 171 and 173 out of the total 176 compounds, respectively. Compound with RQ lower than 0.01 for all samples was neglected.

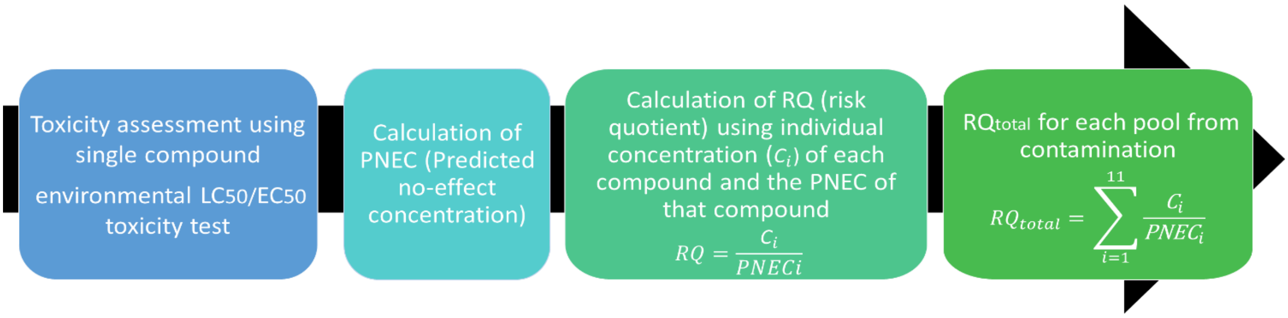


Fig 2. Flow chart depicting the calculation of the RQ for seasonal pools from contamination.
Total RQ is calculated for each sampling event. (Figure 2).

The evaluation of the potential toxicity for sediment was performed in the same way as it was performed for water. Because the RQ calculation is done in accordance with water only, the results were normalized to numbers between 0 and 1 for convenient compare between the pools. Toxicity in sediment is not discussed in this report and is available in the full thesis work of Daniela Abramov (Abramov, 2024).

<u>Spotlight on the prominent micropollutants</u>: For operational purposes, the 20 most influential\ common micropollutants in the pools were identified according to the following criteria: (1) pollutants with RQ higher than 0.01 detected in at least five water samples, (2) pollutants with RQ higher than 0.5 detected in at least one water sample. To this list was added PFOS which has High regulatory interest.

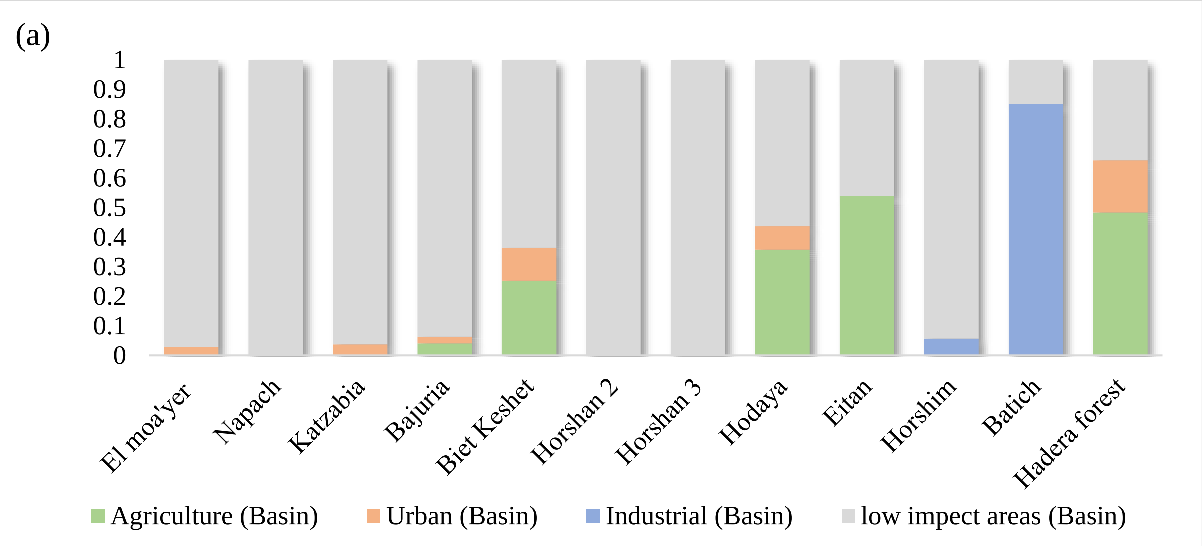
3. Results

detailed presentation of all results (including full tables of contaminants and their toxicity) and in-depth thorough discussion can be found in Daniela Abrahamov's thesis work.

3.1 Characterization of seasonal pools' surrounding

Defining the areas contributing water and micropollutants to the pools was performed by characterizing land uses in drainage basins and 1 km aerial radius around the pools. Low-impact areas, which are the dominant land use in most seasonal pools' surroundings (drainage Basin or 1 km aerial radius), constituted the entire contributing basin of Naphach, Horshan 2 and Horshan 3 pools. Beit Keshet, Hodaya, Eitan and Hadera forest pools are characterized by extensive agricultural areas in their drainage basins between 25.2% in Beit Keshet and 54% in Eitan. In addition, few of the areas of the drainage basins include urban areas between 2.3% in Bajuria and 17.6% in Hadera forest. 85.1% and 5.7% of the Batich and Horshim drainage basins are defined as an industrial area. Low-impact areas were also dominant when looking at the 1 km aerial radius around the pools Beit Keshet, Hodaya, Eitan and Hadera forest pools are characterized by extensive agricultural lands between 26.8% in Horshim and 68.8% in Eitan. In addition, the 1 km aerial radius areas include urban areas between 2.6% in Beit Keshet and 2.7% in El Mo'ayer and 13.8% in Batich. 8% of the area of the Batich and 5.2% of the Horshim buffer zones is defined as an industrial area (Figure 3).

Although the form of measurement had a substantial effect on the size and shape of the contributing areas, in most pools the analysis method only slightly changed its land use distribution(Figure 3). But there are exceptions, for example, in Horshim pool, the area of the drainage basin included only a small percentage of an industrial area, but within an aerial radius of 1 km there are also urban and agricultural areas. Due to the minimal difference between the methods, further analysis and calculations were done based on the drainage basin method.



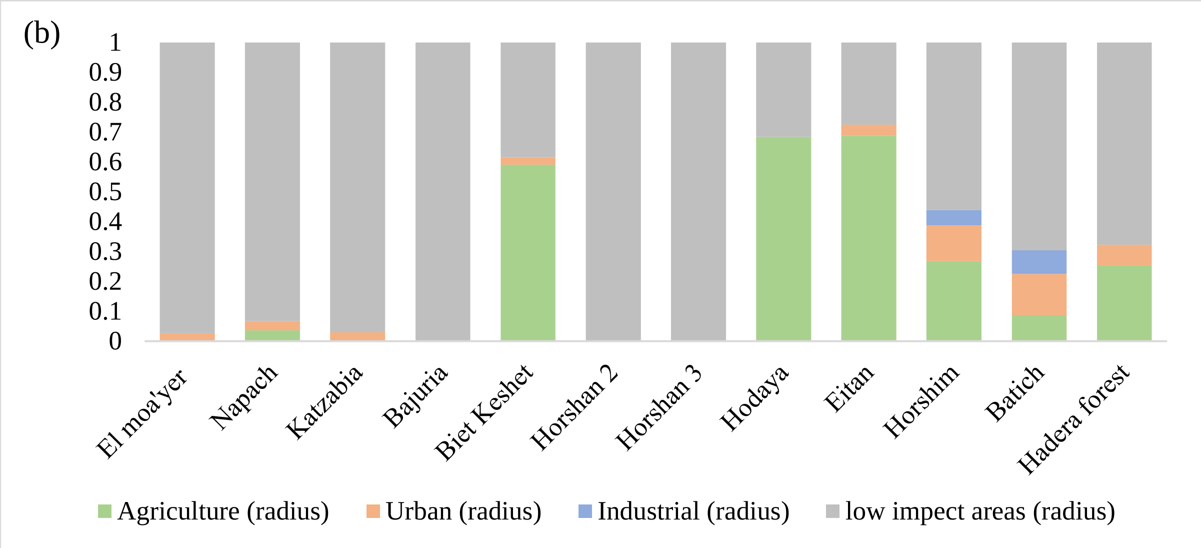


Fig 3. Land use distribution in (a) drainage basins, (b) 1 km aerial radius around sampled seasonal pool.

3.2 Organic micropollutants

3.2.1 Occurrence of organic micropollutants in sediment samples

A total of 53 micropollutants were identified and quantified in seasonal pools' sediments ranging from 7 in Hadera forest, Horshan3 and El Mo'ayer to 25 in Beit Keshet, which was the only pool in which tobacco products were found. Overall, 45%, 32%, and 23% of detected chemicals were classified as industrial chemicals, Plant Protection Products (PPP's), and Pharmaceuticals and personal care products (PPCPs), respectively (Figure 4). Industrial chemicals were dominant in a substantial part of the pools such as El Mo'ayer (86%), Batich (69%), and Horshim (63%). The most frequently detected industrial chemicals were N,N-Dimethyldecylamine, used as chemical intermediates for the manufacture of quaternary ammonium compounds, amine oxide and betaine surfactants, and Benzyl butyl phthalate (a plasticizer), which were found in 10 (83.3%) and 11 (96.6%) of the pools respectively. Among PPP's, DNP (also used for industrial purposes), and the herbicide Diuron, were found in 4 (33.3%) and 6 (50%) of the pools. Among PPCP's, the preservative Methylparaben was detected in most of the pools (83.3%). salicylic acid, an anti-inflammatory and antibacterial agent was detected in 8 (75%) of all pools.

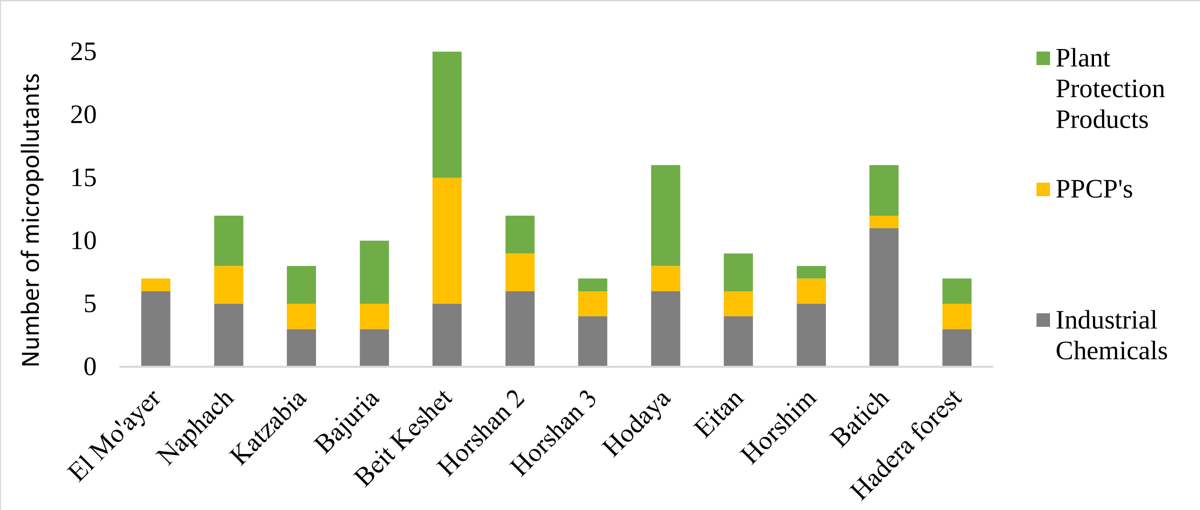


Fig 4. Organic micropollutants, divided by groups, detected in sediments sampled during autumn 2021 from seasonal pools across Israel.

3.2.2 Occurrence of organic pollutants in winter water samples

A total of 116 different pollutants were identified and quantified in 11 seasonal pools as "Beit Keshet" pool didn't have a significant hydroperiod, most likely due to hydrological blockage of runoff (Figure 5). The number of micropollutants ranged from 24 in Horshan2 to 80 in Batich. Overall, 40.5% of the compounds detected in all the pools were PPP's and their decomposition substances, 38% were PPCP's, and 21.6% industrial chemicals. The most frequent compounds detected in all the pools were 2,4-Dinitrophenol (DNP) and Metalaxyl from the plant protection products group, Guanylurea, Saccharine, and Cotinine from the PPCP's group, and Perfluorobutanoic acid (PFBuA) from the industrial chemical group.

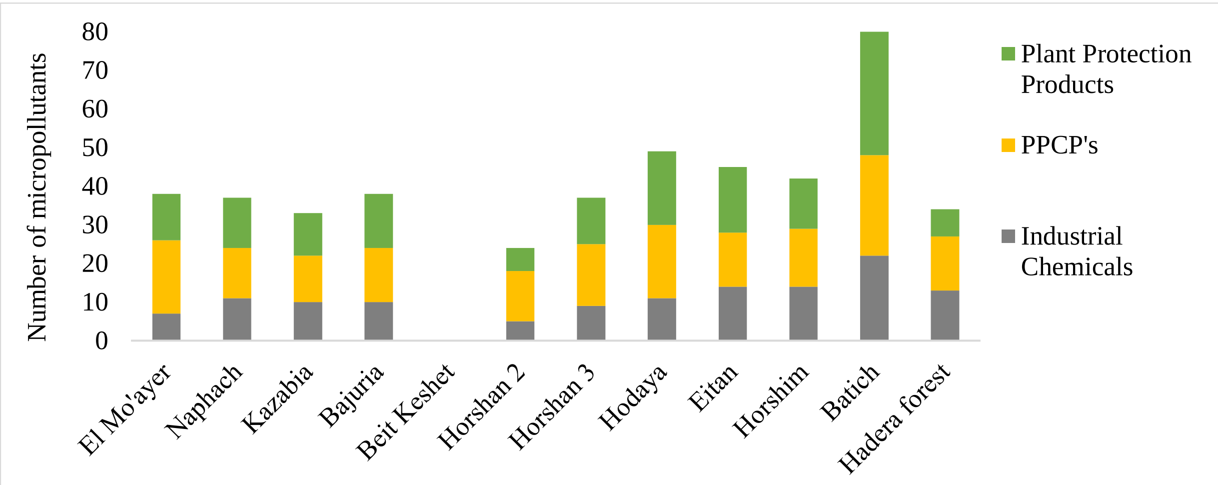


Fig 5. Organic micropollutants, divided by groups, detected in water sampled during winter 2022 from seasonal pools across Israel. .

3.2.3 Occurrence of organic micropollutants in spring water samples

A total of 89 different pollutants were identified in 11 seasonal pools. The number of micropollutants found in the spring water sample ranges from 18 in Hadera forest to 49 in Batich

(Figure 6). 42.7% of the detected compounds were PPP's, 28.4% PPCP's and 37.1% industrial chemicals, 40% of them are PFAS. The most frequently detected compounds in spring sampling were Nicotine from the PPCP's group and Perfluorobutanesulfonate (PFBS) from the industrial chemical group. Both were detected in all sampled pools.

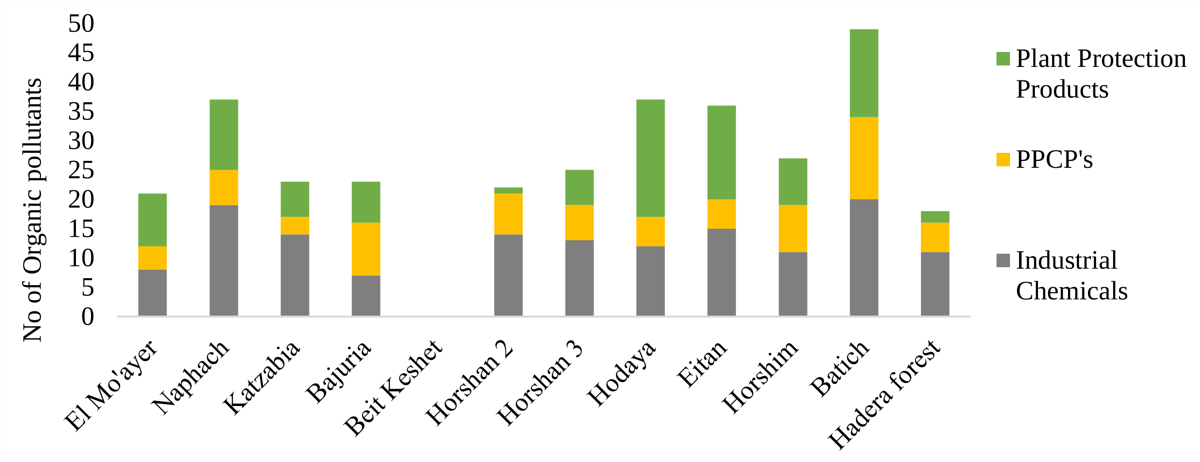


Fig 6. Organic micropollutants, divided by groups, detected in water sampled during spring 2022 from seasonal pools across Israe.

3.2.4 Occurrence of organic pollutants in vegetation samples

A total of 33 different pollutants were identified in the 10 vegetation samples collected during the spring campaign from 8 different seasonal pools. The number of pollutants found in plants ranges from 8 in El Mo'ayer and Horshan2 to 13 in Hodaya (Figure 6). 52.5% of the detected compounds were pharmaceuticals and personal care products, 38.6% plant protection products and their metabolites and Tobacco related compounds and their metabolites, and 8.9% were industrial chemicals. The most frequently detected compounds in plant's tissue were the fungicide Carboxin, and Florfenicol, a common veterinary antibiotic, detected in 90% and 80% of the samples, respectively.

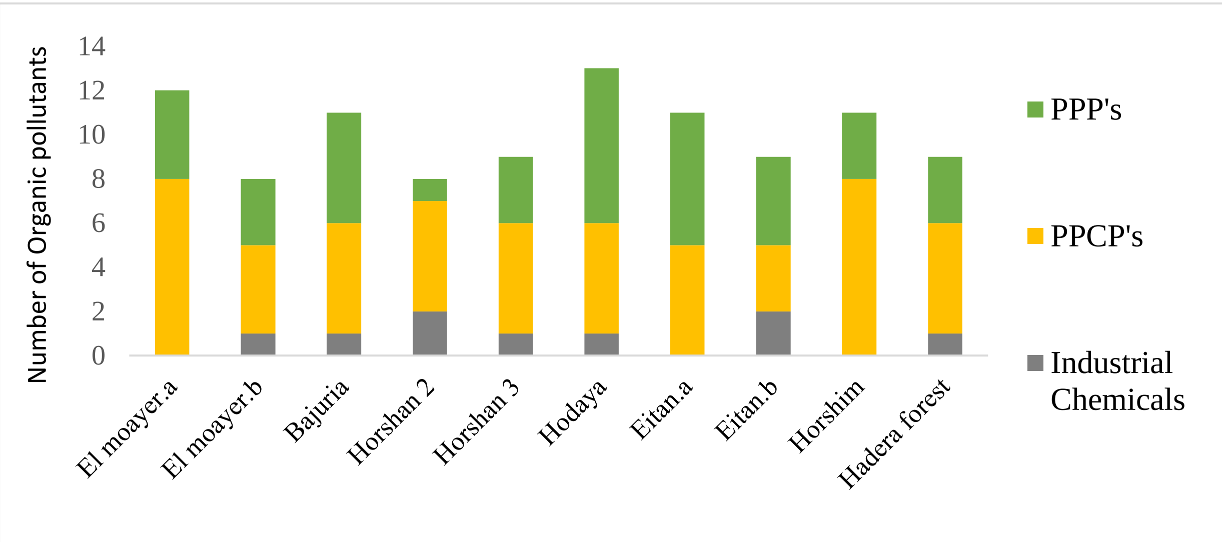


Fig 6. Organic micropollutants, divided by groups, detected in plant tissue sampled during spring 2022 from seasonal pools across Israe.

3.2.5 Temporal changes in the Occurrence and concentration of micropollutants.

Between winter and spring sampling, the total number of pollutants decreased from 116 to 89 (26%) in the range of 8.3% in Horshan2 to 44.7% in El Mo'ayer, with the exception of Naphach pool where the number of the micropollutants remained the same. The number of PPCPs has decreased between winter and spring in all seasonal pools (Figure 7). Plant protection products also showed a temporal decrease with the exeption of Hodaya. Industrial chemicals showed a more complexed pattern and only four pools showed temporal removal: Bajuria, Horshim, Batich and Hadera forest. The other seven pools showed an increased occurrence of industrial chemicals in spring.

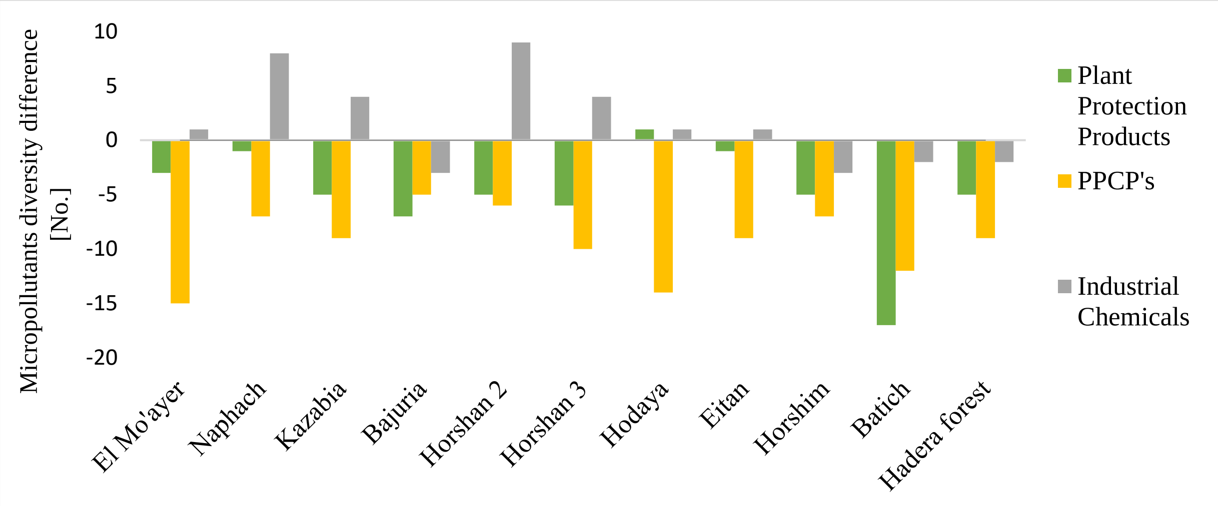


Fig 7. Temporal change in organic micropollutants occurrence between spring and winter water sampling campaigns conducted in seasonal pools across Israel.

The change between winter and spring in the concentration of micropollutants in the pool water was as follow: For the industrial chemicals category, the change differs from decrease by 166% in Bajuria to increase by 98% in Horshan2 (Figure 8). For PPCP's, the change differs from decrease by 462% in El Mo'ayer to increase by 76% in Bajuria. For PPP's, the change differs from decrease by 279% in Horshan3 to increase by 71% in Naphach. In an overall view the decrease in pollutant concentrations is more significant than the increase in trend and percentages. Further information over specific compounds detailed in the thesis.

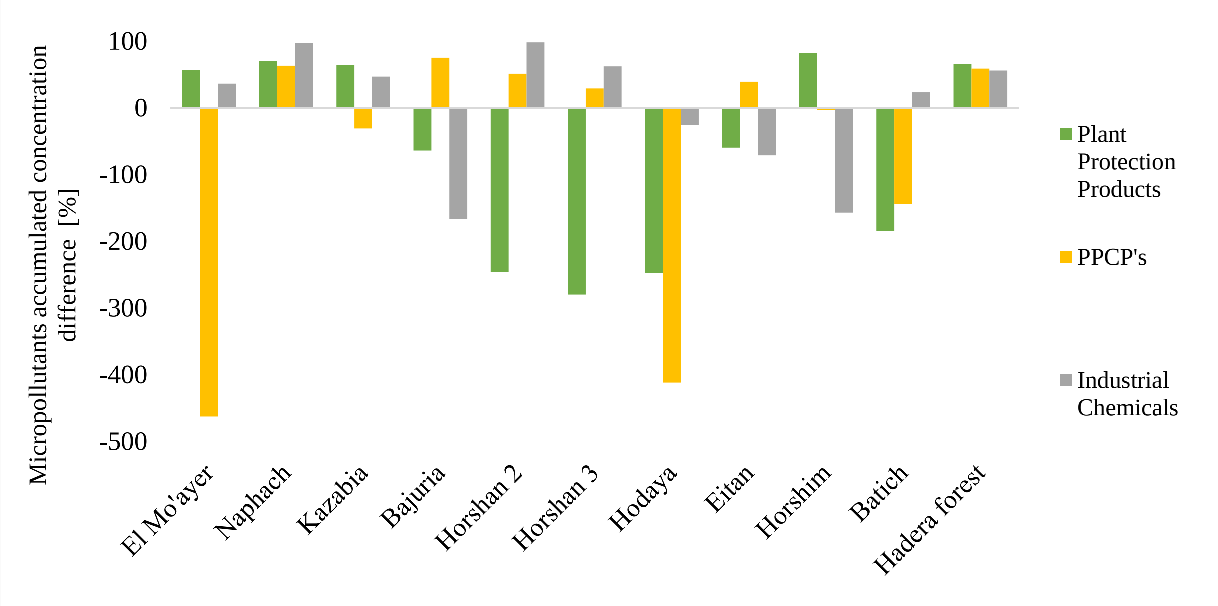


Fig 8. Temporal change in organic micropollutants accumulated concentration between spring and winter water sampling campaigns conducted in seasonal pools across Israel.

3.2.6 Occurrence of organic pollutants by geographic areas

Occurrence of detected pollutants from all categories was plotted against geographical areas (Figure 9). The average number of micropollutants was calculated for every pollutant category (PPCP's, PPP's and industrial chemicals) for each geographical area apart. Some difference was found for the group of PPPs which had elevated occurrence in the southern district.

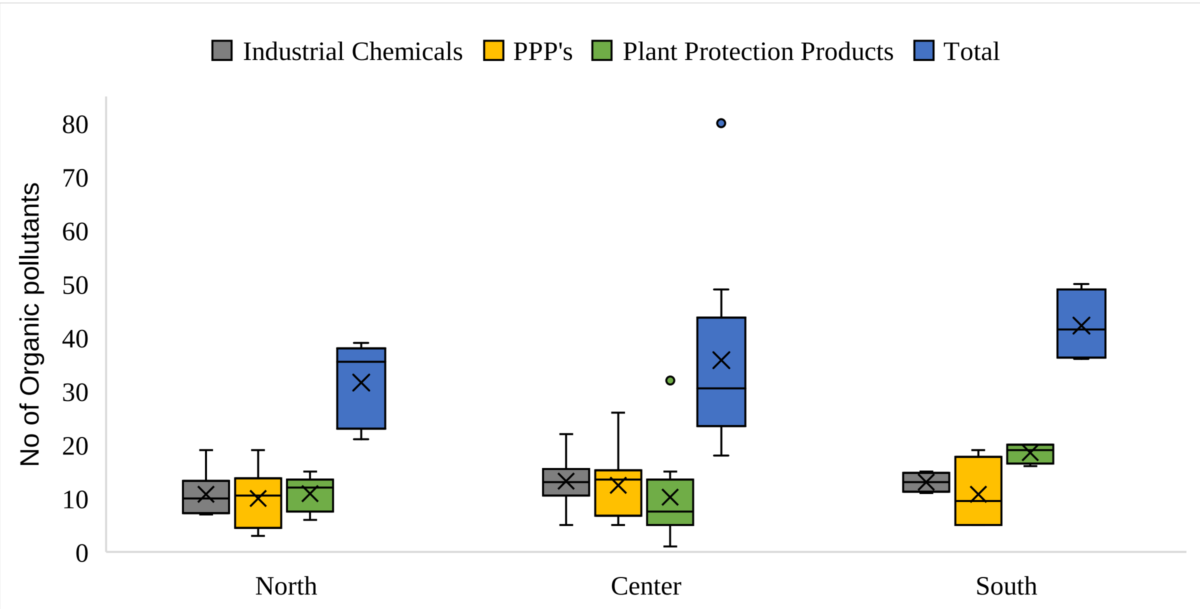


Fig 9. Number of organic micropollutants detections in winter and spring water sampling events in seasonal pools above the detection border line divided by categories and geographic areas.

3.2.7 Calculated toxicity of organic micropollutants in water samples

Accumulated toxicity for algae and crustaceans of all detected compounds were calculated for each pool to retrieve Risk Quotients (RQ) where RQ>1 is considered an unacceptable risk and 0.1<RQ<1 is considered moderate risk (methods chapter).

Algae: In the winter, all pools exhibited a potential risk as none of the pools had an RQ value lower than 0.1. Horshan2 pool showed the lowest RQ (0.16) and Hodaya the highest (90.84). There were several pools exhibiting unacceptable risk to algae, in which an RQ value higher than 1was obtained: Naphach, Bajuria, Hodaya, Eitan, Batich and Hadera forest. The rest of the pools showed an RQ value between 0.1 and 1, which is considered a moderate risk.

In the spring, Horshan3 pool showed the lowest RQ (0.005) and Naphach the highest (21.34. There were several pools in which an RQ>1 value was obtained: El Mo'ayer, Naphach, Bajuria, Hodaya and Batich. The rest of the pools showed a RQ value between 0.1 and 1, apart from Horshan3 which showed negligible risk (RQ<0.01).

crustaceans: In the winter, Horshan2 pool showed the lowest RQ for crustaceans (0.03) and Eitan the highest (3.96). There were two pools, Hodaya and Eitan, in which the RQ was higher than 1, six pools showed moderate risk and three pools showed low risk.

In the spring, Katzabia pool showed the lowest RQ for crustaceans (0.003) and Hadera forest the highest (1.06), which was the only pool with RQ above 1. Moderate risk was obtained in four pools and the rest of the six pools showed low risk. in El Mo'ayer and Katzabia the risk was negligible. Toxicity to algae was higher than toxicity to crustaceans in most pools (Fig 10, table 2).

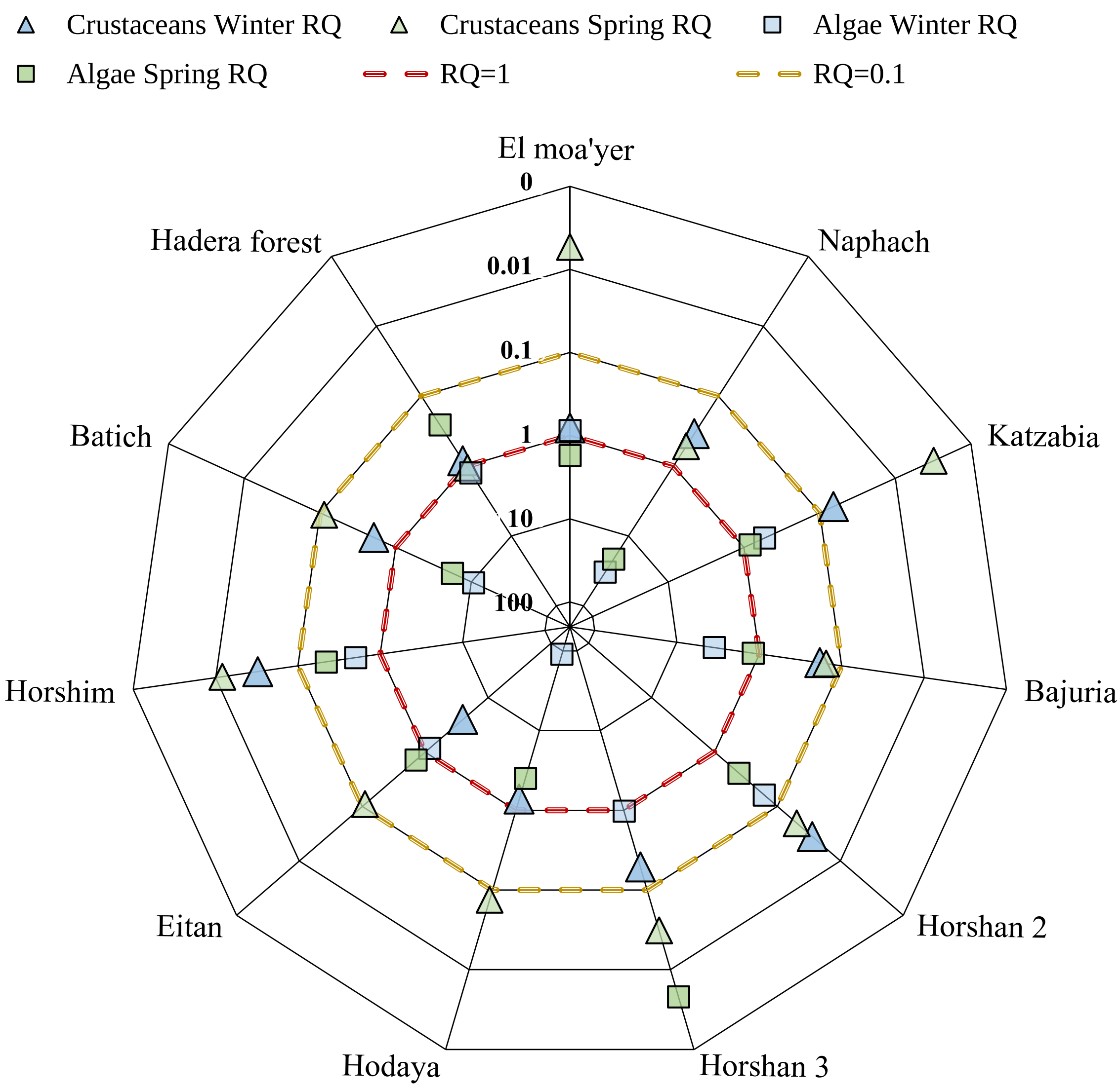


Fig 10. Toxicity potential for algae and crustaceans posed by micropollutants in seasonal pools winter and spring water.

3.2.8 Temporal toxicity changes between winter and spring

Algae: The median RQ for algae was 1.18 in winter and 0.83 in spring, an average reduction of 47%. The RQ value Change ranged from a reduction of 100% to an increase of 151% (Table 2). In El Mo'ayer and Katzabia the RQ value was higher in spring due to elevated concentrations of 2.4-D, an herbicide with high potential toxicity. Horshan2 high elevation in algae toxicity (151%) was mainly due to elevation in the concentration of the toxic Benzophenon 3. Reduction in the potential toxicity of the pools' water between the winter and spring sampling (a period of approximately two months) was driven mainly by the reduction of plant protection products concentrations, specifically herbicides. The most noticeable example was Hodaya pool where the RQ value in winter was composed of mainly Diuron (66.66) and Oxyfluorfen (23.68), that are found jointly in commercial products such as Oasis (Tapazol). In the spring, the contribution of

Diuron to the risk dropped to 0.65 and Oxyfluorfen was not detected. This reduction is probably due to the application period which is before or after the first rain, meaning that the major drift of these compounds occurs during the winter. Yet although the toxicity level drop, this pool suffers from species lost (Yehonatan Bar-Yosef, Personal communication).

Crustaceans: The median RQ for crustaceans was 0.76 in winter and 0.08 in spring, a reduction of 78%. The RQ in most pools was lower in the spring with the exception of Naphach and Hadera, In both pools, the increase in potential toxicity derived from the presence of the industrial chemicals N,N-Dimethyldodecylamine and its TP's and an increase in the concentration of bisphenol a. Overall, the RQ value Changes from a reduction of 99% to an increase of 76%. The most significant reduction was in Eitan pool (3.9), as the RQ value in winter consisted mainly of Difenoconazole (3.89), a fungicide which dropped below the detection limit in spring.

Table 2. Toxicity potential for algae and crustaceans in seasonal pools winter and spring water

Location	Algae Winter RQ	Algae Spring RQ	Temporal change (Algae)	Crustaceans Winter RQ	Crustaceans Spring RQ	Temporal change (Crustaceans)
El Mo'ayer	0.867	1.726	99%	0.810	0.005	-99%
Naphach	32.934	21.344	-35%	0.344	0.520	51%
Katzabia	0.534	0.832	56%	0.066	0.003	-95%
Bajuria	3.542	1.194	-66%	0.184	0.155	-16%
Horshan 2	0.163	0.409	151%	0.028	0.049	76%
Horshan 3	0.976	0.005	-100%	0.194	0.031	-84%
Hodaya	90.837	2.516	-97%	1.380	0.075	-95%
Eitan	1.175	0.717	-39%	3.958	0.111	-97%
Horshim	0.499	0.219	-56%	0.033	0.012	-63%
Batich	10.626	5.585	-47%	0.514	0.114	-78%
Hadera forest	1.261	0.258	-80%	0.839	1.061	26%
Minimum	0.163	0.005	-100%	0.028	0.003	-99%
Maximum	90.837	21.344	151%	3.958	1.061	76%
Average	13.04	3.16	-20%	0.76	0.19	-43%
Median	1.18	0.83	-47%	0.34	0.08	-78%

3.2.9 Connecting land use to potential toxicity.

A multivariate analysis was performed to examine the connections between the land uses and the potential toxicity in the pools. Most of the correlations found were insignificant and did not provide insightful information. A positive/negative correlation found between toxicity for algae versus toxicity for crustaceans. A slight correlation was found (r^2=0.5) between urban land use and toxicity in spring water sampling for crustaceans. Most correlations found were very weak to negligible (r^2=0-0.2) (Figure 11)

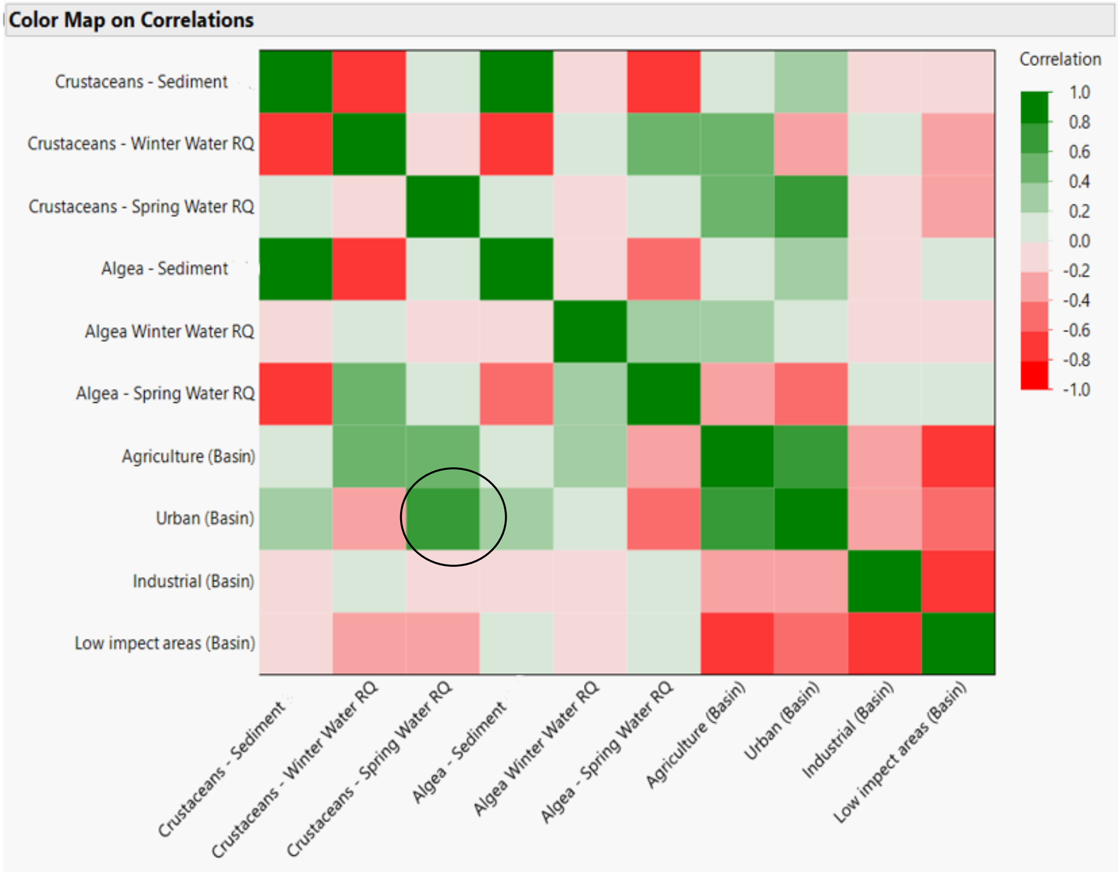


Fig 11. Correlation examination between land use and potential toxicity in sediment, QR for winter water and QR for spring water in seasonal pools. Executed using JMP. A notable correlation is circled.

3.2.10 Occurrence and concentrations of key pollutants

The 20 most notable micropollutants (In terms of occurrence and toxicity) exhibit a wide range of concentrations from less than 1 ng/l (e.g. Acetamiprid, Azoxystrobin, and Anatabine) to thousands of ng/l (e.g. 2.4-D, Diuron, and Bisphenol. A). Compounds differed in detection frequency, distribution patterns, and concentrations. These distinctions are also noticeable between the winter and spring sampling periods (Figure 12). The concentration of prominent industrial chemicals, PFOS, N,N-Dimethyldodecylamine, Bisphenol A and 2-Benzothiazolesulfonic acid generally increased (not for every pool) between winter and spring. Tobacco associated compounds, Anabasine, Anatabine, Cotinine-Hydroxy, were diluted below the detection threshold in most pools by spring sampling. Insecticides also declined in most ponds as Pyrethrin.I declined below the detection limit from the pools and Imidacloprid declined on average but not in all pools. Herbicides showed mixed trends as some compounds increased in distribution and concentrations (Propyzamide, Monuron and 2.4-D) and other decreased (Oxyfluorfen, DNP and Diflufenican). Benzophenon 3 and Ensulizole, both sunscreen agents, were detected in some of the samples. Ensulizole and Benzophenon 3, which is very toxic to Algae, disappeared during spring from pools they were detected in during winter. On the other hand, Benzophenon 3 was detected in significant concentrations during spring in Naphach and Horshan2, pools that it was absent from in the winter.

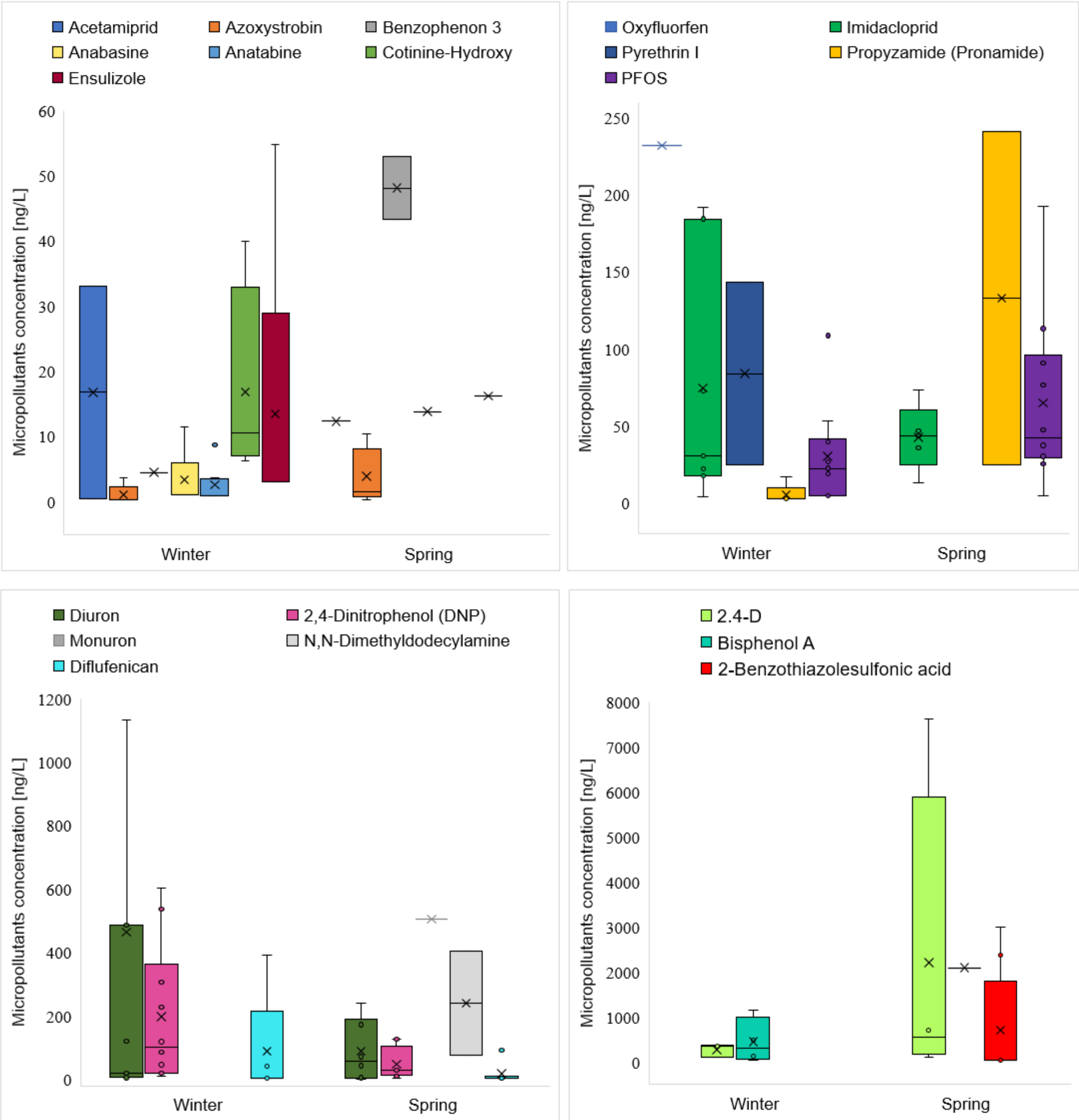


Fig 12. The concentration of prominent micropollutants, originating from industrial, urban and agricultural origins, detected in the water of all pools in winter and spring divided by concentration ranges.

3.3 Biological characteristics of the pools

Invertebrate species were sampled and taxonomically identified by Prof. Frida Ben-Ami's research group. Specific indicators derived from the biological data were used to assess the direct impact of organic pollutants on the pools' biota (KKL-JNF report, Frida Ben-Ami, 2024).

4. Summery

The study aimed to comprehensively explore organic micropollutants in Israeli seasonal pools, examining their occurrence, diversity, and potential ecotoxicity. Mixtures of micropollutants were found in all pools, even in those distanced from direct human influence, and in all mediums (water, sediment, and plant tissue), highlighting the widespread chemical environmental threat. Yet many variations were observed between pools in terms of micropollutant mixtures composition, concentrations, and toxicity, as well as significant temporal changes between winter and spring sampling periods.

Organic micropollutants detected in sediment samples mainly comprised hydrophobic and persistent industrial chemicals, including compounds like Benzyl butyl phthalate and PFOA. Winter water samples exhibited a distribution of micropollutants reflecting runoff quality, with markers like caffeine and nicotine indicating anthropogenic sources (e.g. sewage and wastewater). Spring water samples showed significant changes in micropollutant mixtures composition and concentrations, integrating removal processes of the measured pollutants with additions of micropollutants entering the pools after the winter sampling campaign.

The study raised questions regarding the partitioning of micropollutants between mediums, particularly the ability of sediments and plants to adsorb and remove pollutants from the water column. Limited overlap between mediums might suggest minimal transition from sediment to water, with certain compounds, mainly PPCPs, detected predominantly in vegetation. Although the small number of temporal repetitions of the sampling could have missed fast transitions between the mediums (Figure 13).

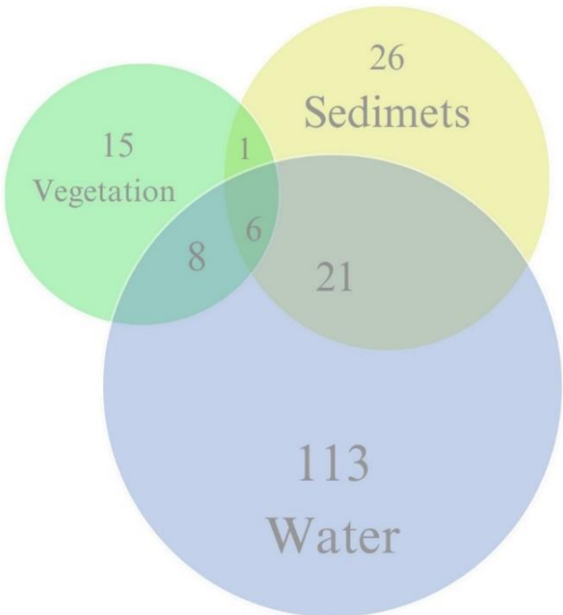


Fig 13. Micropollutants number in each medium in the pools and the overlap of those detected in two of all mediums (in the same pools).

Spatial analysis indicated correlations between urban land use and toxicity to crustaceans (Figure 12), pointing to the strong influence of industrial chemicals. No other significant relationships were found between anthropogenic land uses and potential toxicity, once again highlighting the specific composition of pollutants mixtures in each pool. However, mapping the land uses and detection of micro pollutant combine can be used as a tool for forensic investigation purposes in tracing the route of arrival of pollutants of concern to the pools.

The high diversity in pools characteristics and watershed land uses pose a challenge for drawing broad and spatial conclusions inclusive for all pools that can act as a trajectory for additional pools, not included in the study. Nonetheless, the findings offer insights into pollution sources and risks, providing knowledge on the studied pools, enabling the formation of specific action plans, and creating a basis for further research and environmental management strategies. The conclusions of these investigations can inform authorities in developing measures to reduce pollution in seasonal pools.

5. Environmental implications

The RQ calculations enabled us to assess the role of chemicals in seasonal pools water quality deterioration and clarify the potential source of pollution (Table 3). When calculated risk was significantly elevated, supporting evidence for alteration in pools inhabitants was also found in the biological sampling, suggesting a comprehensive chemical influence threatening the pool's ecological stability (i.e. the case of Eitan and Batich pools). Yet the majority of the results fluctuated across the toxicity benchmark (RQ=1). While this makes it very difficult to derive conclusive understandings of the spatial role and effect of micropollutants, this by no means suggests that the posed chemical risk to the pools is negligible. Even more so, when considering the many additional factors that can alter the success of a pool which may have an additive or synergistic impact on the pools' inhabitants. Hence, at this point, the status of each pool should be examined individually while considering all the necessary factors for a pool success before determining that the toxicity of a certain substance (or a group) is the dominant factor deteriorating it. The relatively low potential calculated risk also presents an opportunity: it suggests that by implementing minor management interventions, it will be possible to reduce the risk values below the thresholds into a confident safe zone, removing the threat of organic micropollutants.

Table 1. Seasonal pools and their dominant chemical pollution type.

Location	District	Pool Area [m^2]	Main organic pollutants source
El Mo'ayer	North	1,708	Urban
Naphach	North	348	Agriculture
Katzabia	North	1,236	Mixed (low)
Bajuria	North	4,232	Agriculture
Beit Keshet	North	8,897	No hydroperiod
Horshan 2	Center	79	Mixed (low)
Horshan 3	Center	687	Mixed (low)
Horshim	Center	225	Urban & Industry
Batich	Center	7,400	Urban & Industry
Hadera forest	Center	42,542	Urban & Industry
Hodaya	South	702	Agriculture
Eitan	South	900	Agriculture

5.1 General (national) operative recommendations

- Creating adsorbing patches (e.g. vegetation or absorbent ground cover) in water entry pathways for mitigating micropollutants before they enter seasonal pools.
- Implementing runoff limiting techniques in contributing intensive agricultural zones (cover crops, buffer strips, etc.).
- Reducing usage of pesticide spraying methods around pools and replacing them with less impacting techniques.
- Raising the awareness of farmers, manufacturers, and local authorities regarding specific compounds used in agriculture and industry that were found to impose a risk to the nearby pool.
- Re-enforcing aquatic plants in pools threatened by pharmaceuticals.
- Establishing a professional-academic committee which will harness the knowledge generated in this work to implement science-based actions for pollutants mitigation.
- Repeated monitoring for pools where a high toxicity risk is found. It can be focused on the 20 most prominent compounds that were determined in this work (Figure 12 & Table 4) with specific adjustments of the monitoring according to the pool surrounding and past findings if needed.

5.2 Pool-specific recommendations

- Explore possible human interference in water supply/pathway to Beit Keshet pool.
- Restrict entrance and activity of cows in Horsha2&3 pools.
- Identify the sources of herbicides (mainly Diuron, Diflufenican, and 2,4-D) which inflicts high algae toxicity in Naphach, a relatively remote and isolated pool.
- For the Batich pool, which is fed by runoff from the nearby industrial area, there is a need to increase enforcement on the adjacent industries to reduce the arrival of industrial leachates. The main measures are covering operational areas and collecting contaminated runoff for disposal through sewage systems (as required by Israeli law).
- In Eitan pool, most of the toxicity to crustaceans was caused by Difenoconazole in the winter mainly. The source of the compound should be monitored and its arrival in the pool should be prevented.
- In Hodaya pool, most of the toxicity to algae was caused by Diuron and Oxyfluorfen, potentially damaging primary production. The source of the compound should be monitored and its arrival to the pool should be prevented.
- It is recommended to resample Hadera forest pool for bisphenol A. If necessary, locate the source of the compounds and act to prevent exposure.

A recommended proceeding work for this study is a study focusing on the establishment of a robust work protocol for the monitoring and management of micropollutant mixtures in seasonal pools (based on the presented findings) which can be used for monitoring additional pools and measuring the success of implemented mitigation practices.

Publications: Preliminary results of the study were presented in SETAC Europe 33rd annual meeting in Dublin, Ireland on the 30/04/23-04/05/23. A concluding scientific manuscript is in advanced preparation.

Table 4. A list of the dominant 20 micropollutants identified in this research with their group and use definition and average concentrations.

Compound	Category	Uses	Average concentration (ng/l)		
			Sediment	Water	Plant
Acetamiprid	Plant protaction products	Insecticide	2.4	2.1	0.0
Azoxystrobin	Plant protaction products	Fungicide	0.2	1.3	0.1
Diuron	Plant protaction products	Herbicide	2.6	256.3	4.9
2.4-D	Plant protaction products	Herbicide	0.0	440.5	0.0
Diflufenican	Plant protaction products	Herbicide	0.1	25.6	0.0
2,4-Dinitrophenol (DNP)	Plant protaction products	Herbicide	22.2	107.0	3.6
Oxyfluorfen	Plant protaction products	Herbicide	0.0	21.1	0.0
Imidacloprid	Plant protaction products	Insecticide	4.3	33.5	0.3
Bisphenol A	Industrial Chimicals	Used to make polycarbonate plastic	0.0	177.9	0.0
Benzophenon 3/ Oxybenzone	PPCP's	Sunscreen agent	0.0	4.8	0.0
Anabasine	PPCP's	Tobacco related compounds	62.9	2.1	0.0
Anatabine	PPCP's	Tobacco related compounds	3.2	2.1	0.0
Cotinine-Hydroxy	PPCP's	Tobacco related compounds	0.0	3.8	0.0
Monuron	Plant protaction products	Herbicide	0.6	45.9	0.0
N,N-Dimethyldodecylamine	Industrial Chimicals	Solvent. Used in making rubber, textiles, drugs, and other chemicals.	2.1	43.7	0.0
Pyrethrin I	Plant protaction products	Insecticides	57.2	15.3	0.0
Propyzamide (Pronamide)	Plant protaction products	Herbicide	0.0	13.4	0.5
2-Benzothiazolesulfonic acid	Industrial Chimicals	Corrosion inhibitors and surface-active chelating agents for mineral processing	0.0	513.3	0.0
Phenylbenzimidazole sulfonic acid/ Ensulizole	PPCP's	Sunscreen agent	0.0	6.1	0.0
PFOS	Industrial Chimicals	Commonly used in non-stick and stain-resistant consumer products, food packaging, fire-fighting foam, etc.	6.5	44.7	0.0

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