In vitro bioanalytical assessment of the occurrence and variation of nine bioactivities in a drinking water treatment plant in Korea

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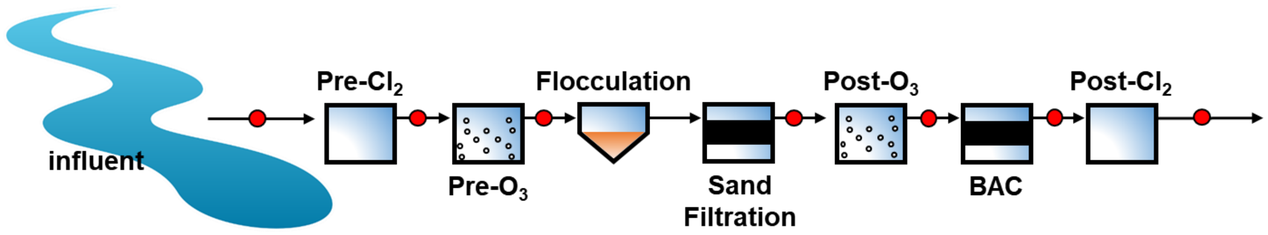
Abstract

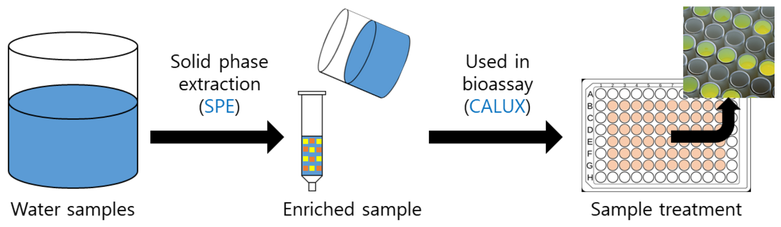
Organic micropollutants in drinking water can pose a public health risk. Chemical analysis alone cannot capture the full range of contaminants or assess their associated risks, promoting the growing use of bioanalytical tools as a complementary approach. This study assessed a drinking water treatment plant in the Nakdong River basin, Korea, using in vitro bioassays targeting nine endpoints. The highest estrogen receptor (ERα) activity was observed in the influent and significantly decreased throughout treatment. Bioactivities related to xenobiotic metabolism (PAH, PPARγ, and PXR) and oxidative stress response (Nrf2) initially increased during pre-oxidation but decreased in later treatment stages. An increase in p53 activity was also noted during treatment. Both season and treatment processes were found to affect the bioactivity variation for most endpoints, based on correlation analysis. The bioactivities observed were consistent with those reported for treated drinking waters in other countries. PAH, PPARγ, PXR, and Nrf2 activities in the final treated waters exceeded some effect-based trigger (EBT) values, indicating potential risks, although uncertainty remain regarding the EBT values for PPARγ and Nrf2. The calculated additive toxicity (CAT) from volatile disinfection byproducts contributed 1.0-2.4% of the measured bioactivities. This study highlights the importance of monitoring of bioactive chemicals to safeguard public health and ecosystems, underscoring the value of in vitro bioassays in water quality assessment.

Research highlights

1. Monitoring of a full-scale DWTP using in vitro bioassays with nine endpoints
2. ERα activity significantly decreased during pre-oxidation treatment processes
3. PAH, PPARγ, PXR, and Nrf2 activities exceeded some EBT values in treated water
4. Seasonal and treatment process variations influenced the bioactivity levels
5. Non-volatile DBPs mainly contributed to Nrf2 and p53 activities, not volatile DBPs

Materials & Methods





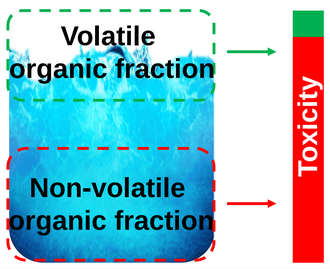
AR	PAH	Nrf2
ERα	PPARγ	Genotoxicity
TRβ	PXR	Cytotoxicity

$$BEQ_{bio} = \frac{EC_{(reference\ compound)}}{EC_{(sample)}}$$

- A DWTP (540,000 m3/day), located downstream of the Nakdong River basin.
- Pre-chlorination (Pre-Cl2) ➔ Pre-ozonation (Pre-O3) ➔ Flocculation & sand filtration (SF) ➔ Post-ozonation (Post-O3) ➔ Biological activated carbon filtration (BAC) ➔ Post-chlorination (Post-Cl2).
- April, May, July, and August of 2021, with grab samples.
- The increased presence of algae (mainly Microcystis) and elevated DOC levels in the influent ➔ Higher chlorine and ozone doses were applied in the summer (July and August).
- Filtration ➔ Solid phase extraction (SPE) ➔ CALUX bioassay.

Table 1. Operational conditions and water quality parameters of the DWTP.

Sampling time	Treatment process		DOC, mg/L	UV_{254}, cm^{-1}	pH
April. - May. 2021	Influent	River water	2.9 ± 0.0	0.057 ± 0.000	8.2 ± 0.1
	Pre-chlorination (Pre-Cl_2)	2.0 - 2.5 mg/L	3.1 ±0.1	0.048 ± 0.014	8.0 ± 0.1
	Pre-ozonation (pre-O_3)	0.4 - 0.8 mg/L	3.0 ± 0.0	0.042 ± 0.004	7.9 ± 0.0
	flocculation/Settling followed by Sand Filtration (SF)	35 - 40 mg/L (Alum)	1.7 ± 0.1	0.020 ± 0.001	7.4 ± 0.1
	Post-ozonation (Post-O_3)	0.4 - 0.8 mg/L	1.6 ± 0.0	0.015 ± 0.001	7.3 ± 0.0
	Biological activated carbon (BAC)	22 - 25 min (EBCT)	1.3 ± 0.1	0.011 ± 0.001	7.3 ± 0.1
	Post-chlorination (Post-Cl_2)	0.9 - 1.0 mg/L	1.1 ± 0.2	0.011 ± 0.001	7.0 ± 0.1
July. - August. 2021	Influent	River water	3.8 ± 0.1	0.090 ± 0.014	8.3 ± 0.4
	Pre-chlorination (Pre-Cl_2)	4.5 mg/L	4.0 ± 0.1	0.084 ± 0.023	8.1 ± 0.2
	Pre-ozonation (pre-O_3)	1.2 mg/L	3.9 ± 0.0	0.086 ± 0.014	8.0 ± 0.1
	flocculation/Settling followed by Sand Filtration (SF)	40 - 60 mg/L (Alum)	2.0 ± 0.4	0.029 ± 0.006	7.6 ± 0.1
	Post-ozonation (Post-O_3)	1.2 mg/L	1.8 ± 0.1	0.018 ± 0.000	7.5 ± 0.1
	Biological activated carbon (BAC)	18 - 20 min (EBCT)	1.3 ±0.2	0.010 ± 0.001	7.7 ± 0.2
	Post-chlorination (Post-Cl_2)	1.0 - 1.4 mg/L	1.1 ± 0.2	0.010 ± 0.001	6.8 ± 0.1



- To assess the bioactivities induced by volatile DBPs, CAT values were calculated using the measured concentrations and potency information.
- Potency was determined based on $EC_{IR1.5}$ values for each volatile DBP.
- $EC_{IR1.5}$ values were available only for Nrf2 p53 activity in the existing literature ➔ The analysis was focused on these two endpoints.

$$Potency = \frac{EC\ (reference\ compound)}{EC\ (detected\ DBP)}$$

$$CAT = \sum Potency \times [detected\ DBP]$$

Results & Discussion

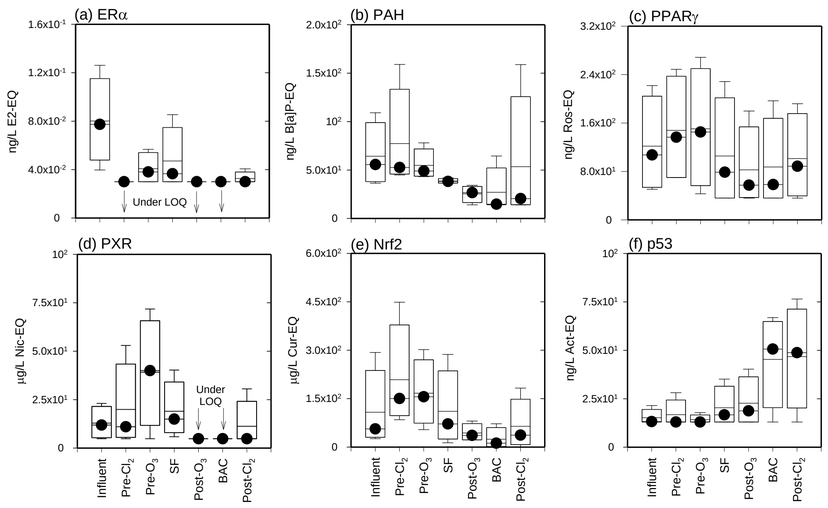


Figure 1. Concentrations of bioactive chemicals in the drinking water treatment plant, monitored over the experimental period. Concentrations are expressed as bioanalytical equivalent concentration (BEQ) of the reference compounds for each bioassay. Bars represent the lower 9% and upper 91% quartile range of the data. Dots within bars indicate the median, and lines represent the mean of the measured data for the samples taken four times. If a value below LOQ is measured in all measurements, it is labeled Under LOQ.

- AR, TRβ, and cytotoxicity are not shown ➔ Generally below the limit of quantification.
- The pre-oxidation processes removed 61% of ERα activity.
- PAH, PPARγ, PXR, and Nrf2 activities increased ➔ Subsequently decreased by 51-93% ➔Slight increase due to post-chlorination process.
- p53 activity showed a continuous increase.
- The observed changes in bioactivities may be attributed to the formation or removal of various non-volatile oxidation/disinfection byproducts during different treatment processes.

- Bioactivity levels in influents and effluents were generally lower than those in WWTP effluents and river waters.
- The xenobiotic metabolism-related effects showed a decreasing trend from WWTP effluents to treated drinking waters.
- Bioactivity in this study similar to that from other countries or regions.
- A comparison with EBT values
  ➔ Hormone receptor-mediated bioactivities were well controlled
  ➔ PAH, PPARγ, PXR, and Nrf2 activities were at or above some EBT values ➔ Indicating potential risks or suggesting that certain EBT values may be overly conservative

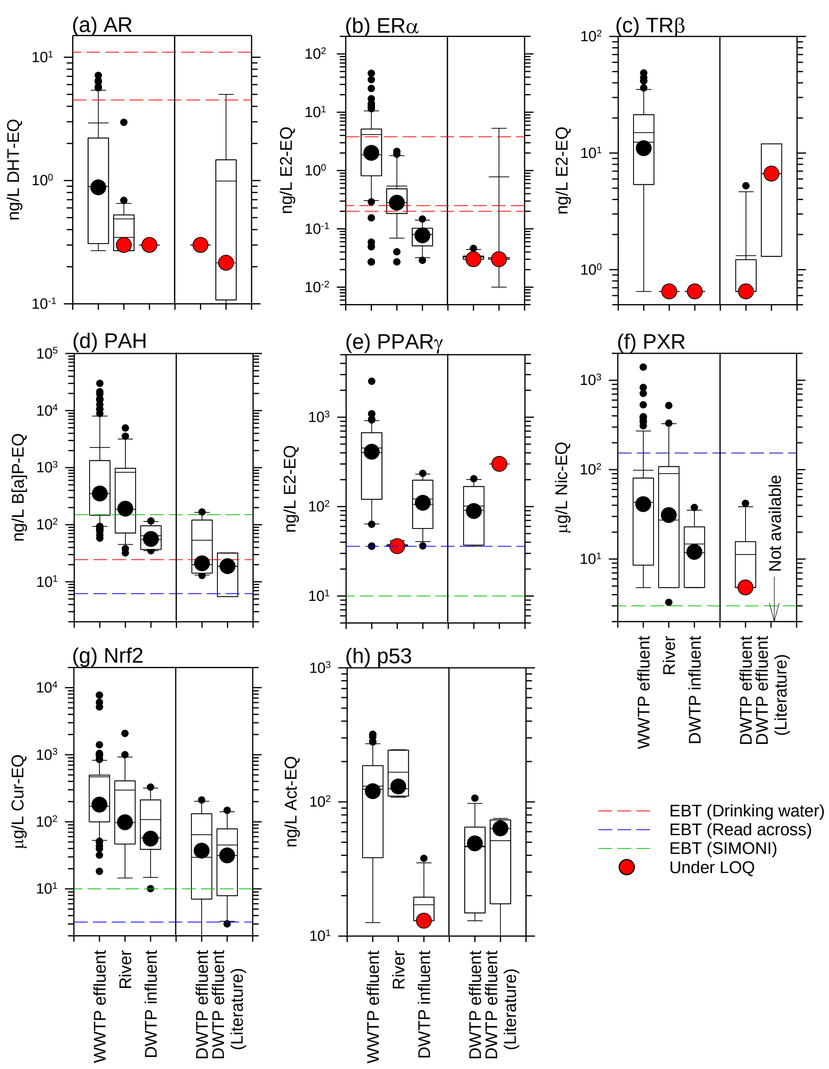


Figure 2. Comparison of the bioactivities in samples measured in this study with those reported in the literature. Bars show the lower 9% and upper 91% quartile ranges of the data, with dots marking the median values, and lines indicating the mean of the measured data. Red dots indicate median values below the LOQ. Horizontal lines denote the effect-based trigger (EBT) values from various sources: red for drinking water, blue for the Read-across approach, and green for the SIMONI approach. When no relevant literature value exists, it is indicated as 'Not available'.

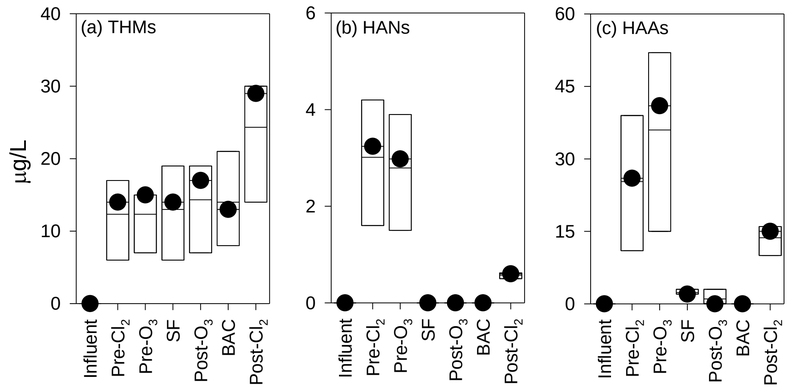


Figure 3. Concentration of disinfection and oxidation byproducts (DBPs) across the drinking water treatment plant processes. Analysis was conducted for three sampling events (May, July, and August, 2021).

- Significant increase in THMs following the pre-chlorination ➔ Remained stable ➔ Increase in the post-chlorination.
- The HANs and HAAs also increased after pre-chlorination ➔ Reductions during the flocculation/sand filtration stage ➔ Slight increase in the post-chlorination.

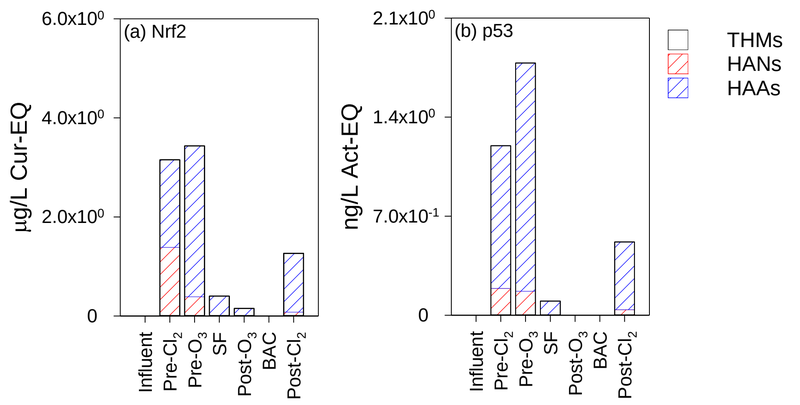


Figure 4. Concentration of calculated additive toxicity (CAT) values for Nrf2, and p53 endpoints. Bars are not shown for influents and certain other cases where DBP concentrations were below LOQ. The mean CAT values for THMs, HANs, and HAAs are shown in different colors.

- CAT values were attributed to HAAs, with THMs contributing insignificantly.
- CAT values for volatile DBPs accounted for up to12% of the total bioactivities, suggesting that non-volatile DBPs pose significantly greater risks.

Acknowledgement

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