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Title: Endocrine and metabolic disruptor effects of wastewater treatment plant effluents from conventional and membrane biological reactor systems: first results

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Abstract

Effluents from municipal wastewater treatment plants (WWTPs) constitute a very complex matrix that can include numerous known and unknown emerging contaminants. To date, the treatment units that make up the plants are not designed to treat most of these contaminants and among these the so-called endocrine disruptors compounds (EDCs), which are natural or artificial chemical compounds (industrial chemicals, veterinary drugs, pesticides and food additives) capable of influencing the correct functioning of the endocrine system in humans as well as in animals (Thacharodi et al., 2023). EDCs interact with nuclear receptors that regulate various physiological processes such as cell development, differentiation, proliferation and metabolism. Furthermore, EDCs combinations can produce additive/synergistic effects, even when each chemical is present at low doses that individually do not induce observable effects. This study aimed to analyze the toxicological characteristics of treated wastewater by comparing the differences between effluents from plants using conventional activated sludge biological technology and advanced membrane treatment effluents (MBR), using a battery of biological tests. For this purpose, the raw influent and treated effluents of four WWTPs operating in the Sicilian territory were sampled, two conventional plants serving the municipalities of Carini and Ribera, the first with nitrification and denitrification treatments for the removal of nitrogen, the second only with organic substance removal treatment, and two MBR plants, serving the municipalities of Riesi and Sciacca, both with nitrification and denitrification treatments for nitrogen removal. Bioanalysis tools, in addition to the physicochemical assessment of water quality, can provide further information on the toxic effects of pollutants present in wastewater discharges by considering the entire mixture of contaminants (cocktail effect) (Shrivastava et al., 2017; Biasiotto et al., 2016). The influent samples (INF), taken downstream of the screening unit, and the treated wastewater samples (EFF), taken downstream of the biological treatment, were extracted and concentrated using solid phase extraction (SPE - Oasis HLB Glass Cartridge). Extracts (with 3x higher concentration factor) were analyzed for endocrine disrupting and metabolic bioactivity using two cell culture assays for estrogenic and obesogenic activity. The results of the analyzes, reported in Figures 1 and 2, showed that all extracts of the influent sewage samples led to an increase in the proliferation rate of estrogen-sensitive MCF-7 breast cancer cells, while no effect was observed for the extracts of treated effluents. Furthermore, no effect was observed in estrogen-negative MDA-MD-231 breast cancer cells confirming the estrogen receptor role in the

effects observed in MCF-7 cell line. This highlights that cleansing treatments, both conventional and advanced, appear effective in withdrawing estrogen-mimicking agents.

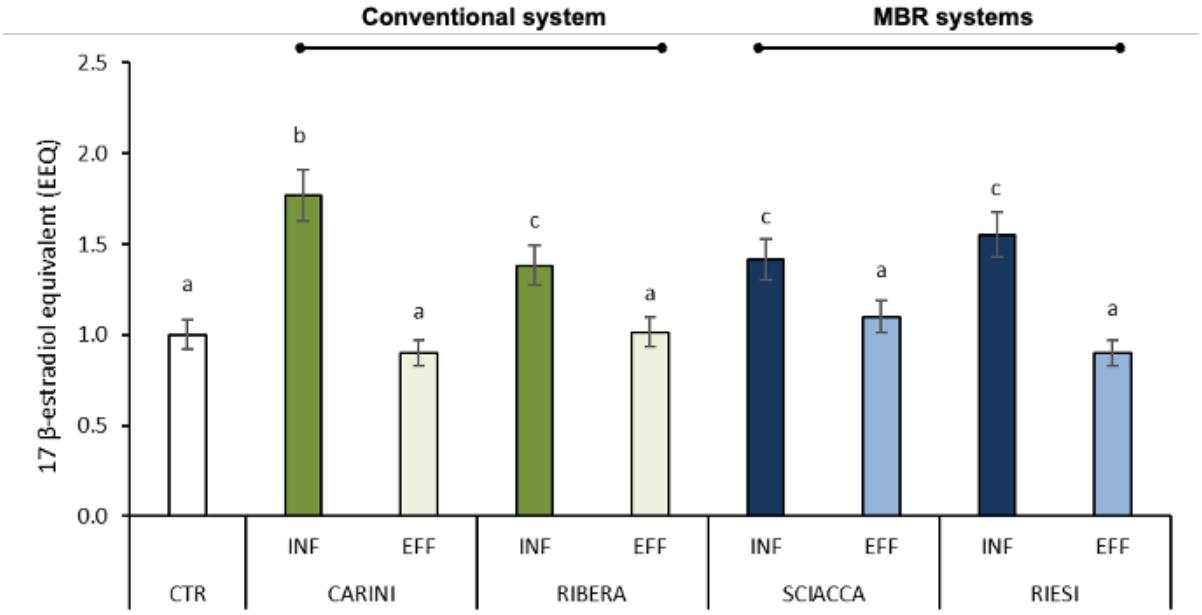


Figure 1 – Estrogenic activity (E-assay) of wastewater extracts on MCF-7 (breast cancer cell line estrogen-responsive). CTR represents a distilled water sample extracted in the same conditions of wastewaters. Means with the same letter are not significantly different from each other ($p > 0.05$)

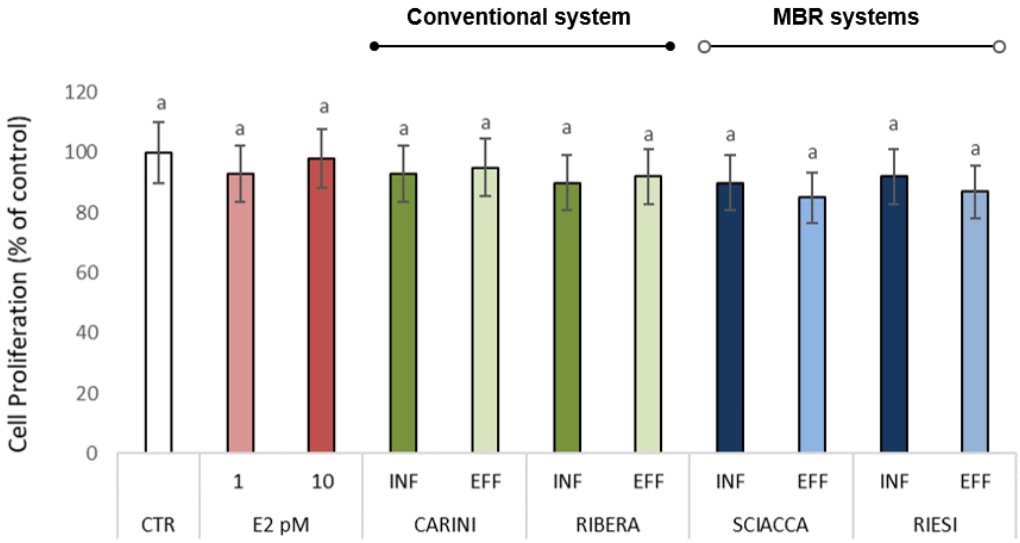


Figure 2 – Effects of wastewaters extracts on MDA-MB-231 breast cancer cell line estrogen-negative. CTR represents a distilled water sample extracted in the same conditions of wastewaters. 17β-estradiol (E2) was used to confirm the lack of estrogen receptor. Means with the same letter are not significantly different from each other ($p > 0.05$)

Exposure to EDCs has been causally linked with obesity in model organisms and associated with obesity occurrence in humans. We then evaluated the effects of wastewaters extracts to promote *in vitro* adipogenesis and lipid accumulation (obesogen effect). At this aim, using an experimental model of preadipocytes differentiation we studied the *in vitro* effects of the wastewater samples to elicit a predisposition to obesity. As showed in figure 3 all extracts from influent samples were able to induce lipid accumulation in 3T3-L1 murine adipocytes and this effect was associated to the presence of compounds with obesogen activity. Finally, the test results highlighted that, while the extracts of the treated effluents taken downstream of plants with conventional treatments showed partial effects, although not significant, on the induction of preadipocytes differentiation (Fig. 3), this was not observed for the effluent extracts from the MBR systems (Fig. 3). This circumstance appears significant as it is symptomatic of a good efficiency of removal of metabolic interfering compounds.

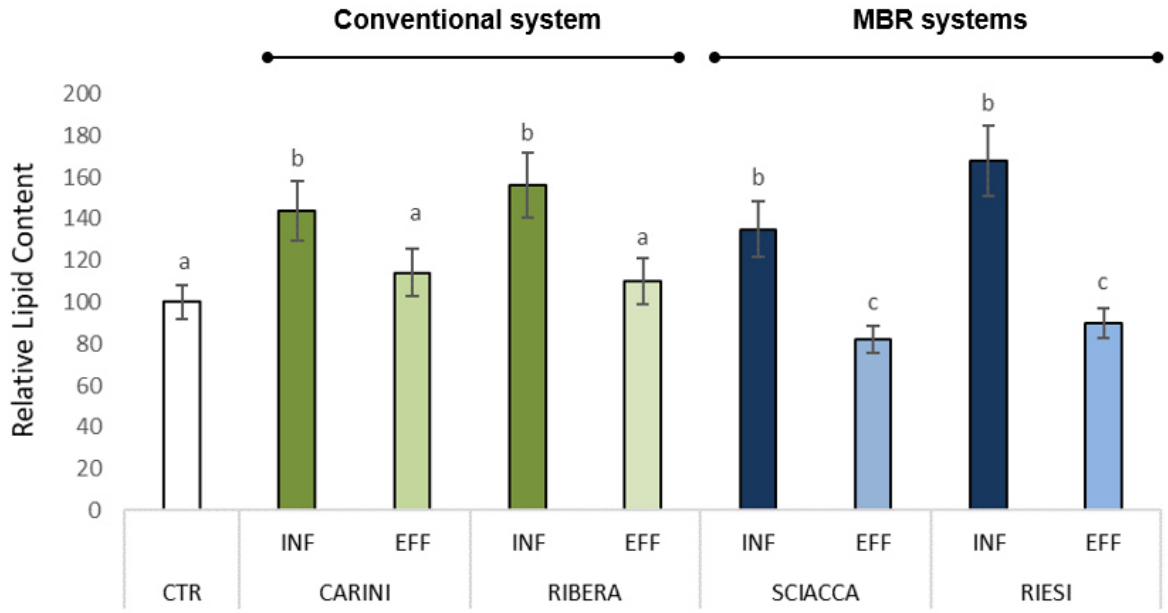
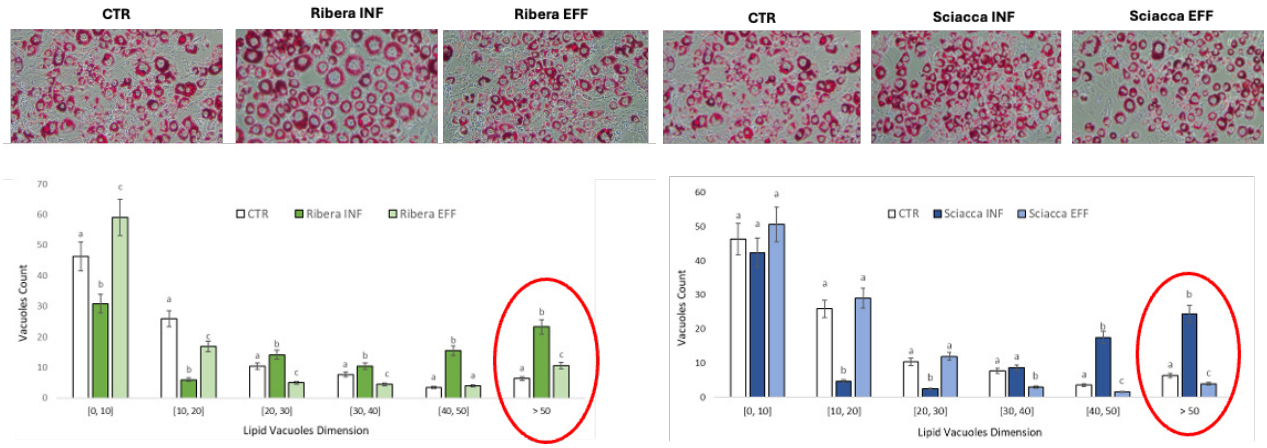


Figure 3 – Intracellular lipid content measured by Oil Red O staining. The lipid droplets accumulated in mature differentiated 3T3-L1 cells were quantified at 490 nm using a microplate reader. Means with the same letter are not significantly different from each other ($p > 0.05$).

However, morphometric analysis of lipid vacuoles dimension (Figure 4), revealed increased vacuoles size for all the influent samples confirming inducing effects on adipocytes fat accumulation. Wastewaters treatments reduced the effects on lipid accumulation even if MBR systems (Figure 4b) showed values similar to CTR. These results further confirmed improved efficacy of contaminant removal exerted by treatments with advanced MBR system.



a) Conventional WWTP b) MBR WWTP

Figure 4 – morphometric lipid vacuole size analysis in 3T3-L1 mature adipocytes performed with ImageJ. The cells were photographed using an optical microscope (magnification 40X). Means with the same letter are not significantly different from each other ($p > 0.05$).

The results of the investigations carried out confirm that, in general, wastewater treatments reduce agents that disrupt the endocrine system and metabolism, although to varying degrees. In particular, MBR technology shows a removal efficiency even on EDCs compounds greater than that obtainable with conventional treatments.

References

Biasiotto G, Zanella I , Masserdotti A, Pedrazzani R , Papa M, Caimi L, Di Lorenzo D. (2016), *Municipal wastewater affects adipose deposition in male mice and increases 3T3-L1 cell differentiation*,Toxicol Appl Pharmacol. Apr 15; 297: 32-40. doi: 10.1016/j.taap.2016.02.023

Shrivastava P, Naoghare P K, Gandhi D, Saravana Devi S, Krishnamurthi K, Bafana A, Kashyap S M b, Chakrabarti T (2017) *Application of cell-based assays for toxicity characterization of complex wastewater matrices: Possible applications in wastewater recycle and reuse*, Ecotoxicol Environ Saf.; 142: 555-566. doi: 10.1016/j.ecoenv.2017.04.023

Thacharodi A, Hassan S, Hegde T A, Dilip Thacharodi D, Brindhadevi K, Pugazhendhi A (2023) *Water a major source of endocrine-disrupting chemicals: An overview on the occurrence, implications on human health and bioremediation strategies*. Environ Res., 15; 231:116097. doi: 10.1016/j.envres.2023.116097