Caffeine as an indicator of estrogenic activity in source waters

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Environmental impact statement

Environmental Impact Statement: Considering the growing number of emerging contaminants, and assuming that around 800 compounds are known or suspect of acting as endocrine disruptors, there is a growing concern about possible damage caused by these compounds not only in aquatic organisms, but also to human health. Assessing estrogenic activity in both source and drinking waters has become almost routine analysis in a near future, but still poses a tremendous analytical challenge. The use of caffeine as an indicator of estrogenic activity opens a novel approach to optimize not only water screening surveys, but also well-established monitoring programs, thus prioritizing samples to be further tested.

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Caffeine has already been used as an indicator of anthropogenic impacts, especially the ones related to the disposal of sewage in water bodies. In this work, the presence of caffeine has been correlated to the estrogenic activity of water samples measured using the BLYES assay. After testing 96 surface water samples, it was concluded that caffeine can be used to prioritize samples to be tested for estrogenic activity in water quality programs evaluating emerging contaminants with endocrine disruptor activity.

Caffeine is widely consumed due to the presence in several species of plants, coffee, tea, cocoa, herbs and analgesic drugs, acting as a stimulator of the central nervous system.[1] Caffeine is partially metabolized in the human body and is excreted through the urine. Because wastewater treatment plants do not completely remove this compound,[2] and also because of its high water solubility, caffeine can reach natural water system such as surface and underground waters. Thus, the presence of this compound in natural waters is strongly related to anthropogenic activity, and therefore has been used as an good indicator of water quality regarding the presence of sewage.[1-9]

Caffeine levels detected in water bodies are related to the sanitation condition of a region. A study carried out in the Boston Harbor seawater (USA) showed that caffeine was the main contaminant found in this aquatic system, where the presence of raw and treated sewage was consistent with data on the consumption pattern in the region.[3] In surface waters around Madrid (Spain), different concentration ranges measured (675 – 13 167 ng/L) correlated with the distribution of the population in the investigated metropolitan area.[10]

Even in countries with high levels of sanitation, caffeine can still be detected in surface waters, as reported in France (13 – 107 ng/L),[11] Istanbul (Turkey) (21 – 20 427 ng/L),[12] Italy (0.6 – 1 056 ng/L)[13] and USA (2 – 225 ng/L).[14-17]

In Brazil and others countries with poor sanitation condition, the scenario of contamination of water bodies by sewage is severe, especially due to the high input of raw sewage directly into the surface water systems, where caffeine levels spans over up to two orders of magnitude higher than those determined in European countries and USA. Caffeine was found in the range of 174 and 127 092 ng/L in surface waters from São Paulo[19] and between 1 410 and 753 500 ng/L in the city of Curitiba, Paraná.[9] Mean concentration of 219 100 ng/L was measured in surface waters in the State of Rio de Janeiro.[8]

Underground water systems may also be contaminated with caffeine. In the USA, 1231 groundwater samples collected from May 2004 to March 2010 by the California Groundwater Ambient Monitoring and Assessment (GAMA) Program Priority Basin Project, the median concentration of caffeine reported was 170 ng/L, with a maximum concentration of 290 ng/L.[20]

Caffeine is only partially removed in drinking water treatment plants, being the extent of removal related to the type of treatment and the sewage burden in the raw water. Concentrations ranging from 2 to 260 ng/L and 5 to 82 ng/L were determined in source and drinking water, respectively, in France.[21] Concentrations between 10 and 53 ng/L were measured in drinking water in Italy,[13] from 50 to 396 ng/L in Spain,[10,22] from 1 to 181 ng/L in USA[16,17] and in the range of 7 to 108 ng/L in Canada.[23] Mean concentrations of 24 and 23 ng/L were reported for drinking water in France[11] and China,[24] respectively. In Brazil, caffeine in drinking water was reported to occur at concentrations ranging from 47 to 5 845 ng/L.[25]

The occurrence of caffeine has been compared to other traditional microbiological and physico-chemical water quality parameters, and it has been a reliable indicator of pollution in urban aquatic environments.[8] Caffeine has also shown a positive correlation with biochemical oxygen demand (BOD) and nitrate in natural waters.[9] In addition, caffeine could be used as a tracer for the quantification of untreated wastewater input into underground water systems.[26] The presence of this compound has also been considered to be a more effective indicator of the presence of sewage than *E. Coli* due to the fact that caffeine is related exclusively to the human body excretion.[2] The correlation between caffeine and faecal coliforms ($R^2 = 0.45$) showed that caffeine is a promising indicator of recent input of sewage in natural waters.[6]

The purpose of this work was to evaluate the feasibility of using caffeine and other emerging contaminants to predict the estrogenic activity in natural waters sampled in Brazil. A group of 16 compounds was selected to be monitored: caffeine (I), estrone (II), 17β-estradiol (III), estriol (IV), progesterone (V), testosterone (VI), mestranol (VII), levonorgestrel (VIII), 17α-ethynylestradiol (IX), diethylstilbestrol (X), triclosan (XI), bisphenol A (XII), 4-n-octylphenol (XIII), 4-n-nonylphenol (XIV), phenolphthalein (XV) and atrazine (XVI). Ninety-six samples were collected in seven different surface waters sampling sites in the State of São Paulo: Atibaia River, Capivari River, Piracicaba River, Corumbataí River, Cotia River, Sorocaba River and Tanque Grande Reservoir from March 2010 to April 2011.

Using 1 L of sample, the selected compounds were extracted using Solid Phase Extraction (SPE) with OASIS HLB (Waters) and eluted with methanol and acetonitrile. Details of sampling procedures and sample pre-treatment are described elsewhere.[25,28] The quantification was carried with a Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) using an Agilent 1200 Series LC system coupled to an Agilent 6410 Triple Quadrupole mass spectrometer with an electrospray ionization source.[25] *Saccharomyces cerevisiae* bioluminescent bioreporter assay (Bioluminescente yeast estrogen receptor assay - BLYES) was used to evaluate the estrogenic activity (EA) of the same water extracts. This specific bioluminescent strain was previously described[27] and the procedures used in this work were already published.[28,29] Samples extracts were split into two, one part being used for the chemical analysis and the other for the BLYES assay.

The results obtained for the different compounds were evaluated by univariate and multivariate (Principal Component Analysis, PCA) analysis using the Unscrambler 10.3 chemometric software (CAMO, Norway). Figure 1 shows the direct univariate correlation between caffeine concentration and EA. A reasonable correlation (correlation coefficient, $R^2 = 0.4664$) was found.

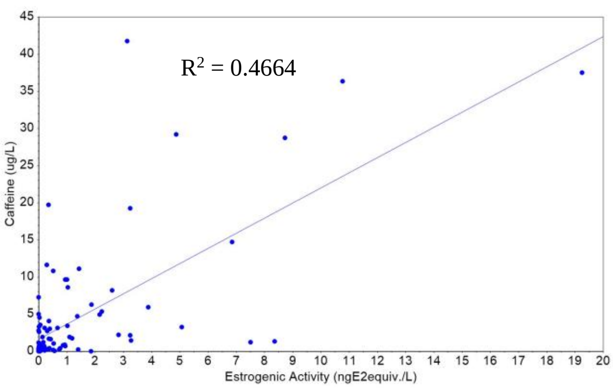

Figure 1. Scatter plot of the concentration of caffeine and estrogenic activity for the 96 tested samples.

Figure 2 depicts the distribution of the loadings for the first and second principal components (which explain together 45 % of the data variability) for the 8 (of the 16) contaminants that were quantified at least once and the measured EA. Again, it is possible to conclude that there is a significant correlation between caffeine concentration and estrogenic activity, considering the closeness of the loading values of these parameters observed for the first and second PC, and the significant variability of the data set they describe. In addition, it is also possible to observe that some compounds considered low inducers of EA may be present in the water without presenting a significant correlation with the measured estrogenic compounds present in the samples, such as XV, XII, II and XIV. On the other hand, estriol (IV) appears to show a significant correlation with EA, as expected.

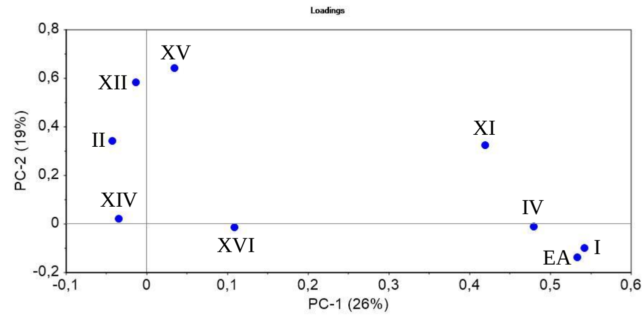

Figure 2. Plot of the loadings resulting from the PCA analysis performed on 9 variables of the data set (8 contaminants concentration and EA).

As already discussed by Bergamasco et al.,[29] the higher sensitivity of the BLYES assay in comparison to the expected activity of each individual compound that was measured could be explained by synergistic effects or by the presence of strong estrogenic substances, such as 17α-ethynylestradiol, which could produce some response even at concentrations lower than the limit of detection of the LC-MS/MS method employed in this study. It is important to emphasize the fact that the BLYES assay is around 200 times more sensitive than the LC-MS/MS in terms of 17β-estradiol (0.01 ng $E2_{equiv.}$/L and 1.8 ng/L, respectively).[25]

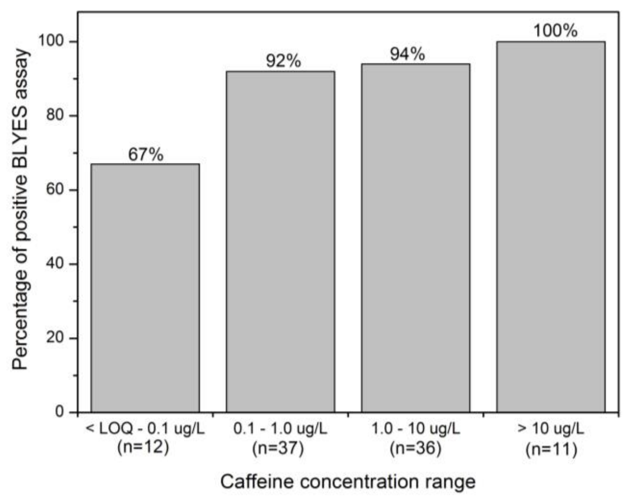

Figure 3. Percentage of source water samples (n) tested positive for the BLYES assay as a function of the caffeine concentration range. Total of 96 samples.

From a total of 12 samples with caffeine concentration between < LOD (0.005 μg/L) and 0.1 μg/L, 67 % presented positive results for BLYES; next, for a total of 37 samples showing caffeine concentration between 0.1 and 1.0 μg/L, 92 % were positive for BLYES; in water samples (n=36) presenting caffeine concentration between 1.0 and 10 µg/L, 94 % were tested positive for BLYES; for the 11 samples showing caffeine concentration above 10 µg/L, 100 % presented positive results for BLYES (Figure 3). Considering all samples that showed caffeine concentration above 1 µg/L (n=47), 96% showed positive results for estrogenic activity using the BLYES assay. This trend is important from both environmental and monitoring perspectives, as caffeine concentration can be used to prioritize sites to be studied. Also, using only caffeine as an indicator of estrogenicity, it is possible verify seasonal periods that deserve more attention concerning endocrine disrupting activity. In summary, high concentrations of caffeine in source water samples indicate high input of sewage, and consequently higher concentrations of compounds such as hormones and other pharmaceuticals that positively respond to the BLYES bioassay.

Conclusions

The correlation observed between caffeine levels and estrogenic activity (EA) strongly suggest that in natural waters, this compound is a powerful candidate to be used as a tracer to screen samples with high probability of showing estrogenic activity in water monitoring programs. However, it is important to mention that at the concentration levels found in the tested waters, caffeine poses no risk to human health neither is it an endocrine disruptor candidate. One advantage to be highlighted is the fact that caffeine can be determined using simple and low cost analytical methods such as SPE HPLC/DAD.[19] The prioritization based upon caffeine levels has a direct effect in lowering the required number of samples to be assessed by a more sophisticated and expensive method.

Although a correlation between caffeine and estrogenic activity was established using the BLYES assay, one should bear in mind that this correlation depends on the type of estrogenic assay used, and a database should be constructed for each different EA assay chosen.

Finally, assuming the possibility of having water quality criteria for EA, it will be possible to use this correlation to define a threshold value for caffeine concentration to predict estrogenic activity with high levels of confidence.

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Notes and references

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