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Influence of Excess Biological Sludge Disintegration Processes on Pharmaceutical Compounds, Hormones and PAH Concentrations from Anaerobic Stabilized Sludge

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Abstract: *This paper shows the influence of certain biological sludge disintegration processes over the concentrations of emerging pollutants, including classes of structurally different compounds such as pharmaceuticals, hormones and polycyclic aromatic hydrocarbons from anaerobic stabilized sludge. Three processes for sludge disintegration were tested: ultrasonic, ultrasonic + electrokinetic and ultrasonic + electrokinetic + microbubble generation. Activated sludge from an urban wastewater treatment plant was used in the experiments. Both pharmaceuticals from the analgesic and antipyretic classes, as well as the identified hormones (estrone) and polycyclic aromatic hydrocarbons (PAHs), were sensitive to the disintegration processes applied, the experimental results showing the decrease of their concentration correlated with the increase of the disintegration energy.*

Keywords*: sludge disintegration, anaerobic stabilization, pharmaceuticals, hormones, PAHs*

1. Introduction

Emerging pollutants, such as pharmaceutical compounds, hormones and polycyclic aromatic hydrocarbons (PAHs) can be found in wastewater and in activated sludge. Recent studies have showed that traces of pharmaceutical compounds from the anti-inflammatory class can be found in the Danube river and its major tributaries in Romania, due to the fact that these compounds can be released without prescription [1]. Similar studies were conducted relating the occurrence of hormones in various environmental components. Hormones from the estrogen class (estrone, estradiol, and estriol) were also detected in the Danube River and in municipal wastewater treatment plants (WWTPs) [2, 3]. PAHs, organochlorine compounds and platinum elements are a type of pollutants that can be found everywhere, in water, wastewater, soil and activated sludge [4-6]. The removal of this type of emerging pollutants from activated sludge in order to improve its characteristic represents a general focus. Sludge disintegration process can be defined as pretreatments classified in mechanical, chemical and biological that are applied in order to improve advanced anaerobic sludge stabilization and in order to remove the unwanted pollutants. Most of the studies have shown that ultrasonic disintegration, electrokinetic disintegration and a combination of ultrasonic + electrokinetic + microbubble generation disintegration processes can be efficient in hormones [7-9] and pharmaceutical compounds [10-12] and even in polycyclic aromatic hydrocarbons removal. The aim of this study was to evaluate the efficiency of some degradation technologies for pharmaceutical (analgesic/anti-inflammatory, antipyretic) compounds (diclofenac, ibuprofen, ketoprofen, piroxicam), estrogenic hormones (estriol, equilline, estrone, 17 β estradiol, 17 α etinilestradiol, 17 α estradiol) and the PAHs (naphthalene, anthracene, phenanthrene, fluoranthene, pyrene, benz(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, indeno(1,2,3-cd)pyrene, benzo(*ghi*)perylene) in WWTP sludge samples.

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2. Materials and methods

In order to determine the sludge disintegration effect over pharmaceutical compounds, hormones and polycyclic aromatic hydrocarbons concentrations from anaerobic fermented sludge, activated sludge from an urban wastewater treatment plant was used within the experiments.

The activated sludge pretreatment prior to anaerobic fermentation by disintegration (ultrasonic, ultrasonic + electrokinetic, ultrasonic + electrokinetic + microbubbles generation) was performed in an experimental model installation with the characteristics:

- Flow rate 0.35 m^3/h;
- Power 1 kW (ultrasonic disintegration) + 0.35 kW (electrokinetic disintegration);
- 1 m^3 sludge tank capacity;
- Ultrasound frequency 20 kHz;
- High voltage download 100 kV, with high frequency pulses 7 kHz.

Anaerobic fermentation experiments were carried out at 36ºC in four bioreactors immersed in a tank, thermostated using a Thermo Fischer Scientific thermostat equipment. Thickened excedentary activated sluge from an urban wastewater treatment plant was used, which was disintegrated (ultrasonic / ultrasonic + electrokinetic / ultrasonic electrocinetic + microbubble generation) at various energies in the experimental disintegration modules.

The pharmaceutical compounds were determined by liquid chromatography coupled with mass spectrometry (LC-MS/MS). The sludge samples were initially lyophilized and afterwards homogenized. A portion of the homogeneous mixture was subjected to ultrasound-assisted extraction, using as an extraction solvent a mixture of methanol and acetone, in a 1:1 ratio. The samples were subsequently centrifuged for phase separation. The obtained supernatant was purified by solid phase extraction using StrataX-type polymeric SPE cartridges and the extract obtained was evaporated to dryness gentle nitrogen stream. The samples tested for pharmaceutical compounds (diclofenac, ibuprofen, ketoprofen and piroxicam) were re-dissolved in mobile phase mixture (methanol and Aq 0.04 acetic acid in a 1:1 ratio) and subjected to LC-MS/MS analysis. These were separated using a Zorbax Eclipse Plus C8 type column (2.1 x 100 mm, 3.5 μm), identified and quantified using a triple-quadruple mass spectrometer (QQQ) detector [1].

The method for hormones determination was based on sludge lyophilization and successive extraction with methanol-acetone by ultrasonication, centrifugation for phase separation and clearing solution. the Analytical procedure was previously developed within a research project [10]. The sludge sample was dried using Christ Alpha 1-2 LD lyophilizer (Martin Christ GmbH, Germany), crushed and sieved (particle size less than 100 μm). The analytes were extracted with 5x2 mL of MeOH and 2x2 mL of acetone by ultra sonication at 30°C, after then the phases were separated by centrifugation (20 min at 3000 rpm). The organic extract was diluted at 100 mL with HPLC grade water and then was purified by solid phase extraction (C18 SPE) using AutoTrace 280 SPE system (Thermo Scientific Dionex). The resulted extract was evaporated to dryness at 45°C after that the residue was dissolved in 1 mL of mobile phase. The analytes were separated by LC-MS (Agilent 1260 - Agilent 6410B) method using electrospray ionization source. The compounds were determined using an LC Zorbax Eclipse Plus C18 column (150 x 2.1 mm, 3.5 μm) at 20°C. The mobile phase was composed by 0.01% ammonium hydroxide and methanol (50/50, v/v) and ruled by LC system with Agilent 6410B Triple Quadrupole detector at a flow rate of 0.2 ml/min [3].

Polycyclic aromatic hydrocarbons were determined according to ISO 13859: 2014 (E) - Soil quality - Determination of polycyclic aromatic hydrocarbons (PAHs) by gas chromatography (GC) and high-performance liquid chromatography (HPLC). The method is based on the extraction of PAHs from soil, sludge / waste in organic solvents (acetone, petroleum ether) by mechanical agitation followed by the concentration of the extract and the removal of interferents by C18 SPE purification. The eluate is concentrated and taken up with a polar solvent. The extract is analyzed by HPLC-FLD in the reverse phase and the PAH compounds are identified and quantified using the external standard method Sludge samples were first lyophilized for 24 h, crushed and homogenized by a mortar and

pestle. In order to extract of PAH, to the dry sludge sample (1g) was added acetone (10 mL) and petroleum ether (5 mL) in a ratio of 2: 1, followed by mechanical stirring 12h. After decanting the solids, the acetone was removed by washing with distilled water (400 mL) twice, in a separating funnel. The extract obtained was dried over anhydrous sodium sulfate (5g). Then the obtained extract was subjected to purification, in order to eliminate the interfering matrix, by eluting the extract onto an aluminum oxide layer (2g), using a separating column (glass) and eluting with petroleum ether (10mL). Then, the solvent was changed by adding 0.5mL of acetonitrile to the eluate and the extract was evaporated in a water bath at 400C at 0.5 mL, and then the whole volume was brought to 1mL with acetonitrile. The organic extract was injected (10 µL) into the Ultimate 3000 liquid chromatograph (Thermo Scientific) equipped with the quaternary pump, autosampler, chromatographic column thermostat and fluorescence detector. The software used for data acquisition was Chromeleon 7.2. The separation was performed on the reverse phase column, Hypersyl Green PAH (dp 5 µm, 250 mm length x 3 mm inner diameter), at a flow rate of 0.9 mL/min with a mobile phase of acetonitrile (A) / water (B). The mobile phase gradient was: 0-3 min 60% A, 3-15 min 60- 100% A, 15-27 min 100% A, and the column balancing time was 9 min. The PAH quantification was performed using the external standard method using linear calibration regressions in the range 0.01-0.4 mg / kg (0.1-4mg / L) with coefficients of determination > 0.998 [13].

3. Results and discussions

Three types of sludge disintegration were tested: ultrasonic, ultrasonic + electrokinetic and utrasonic + electrokinetic + microbubble generation.

3.1. Ultrasonic sludge disintegration

In order to evaluate the effects of ultrasonic disintegration of the thickened biological sludge over the pharmaceutical compounds, hormones and PAH concentrations, samples of anaerobic fermented sludge were collected and analyzed. Table 1 presents the characteristics of anaerobic fermented sludge with ultrasonic biological disintegrated sludge component.

The reaction mixture component formed by thickened biological sludge: thickened primary sludge: fermented sludge / inoculum in a 3:1:2 ratios was first subjected to anaerobic fermentation.

Table 1. Anaerobic fermented sludge with ultrasonic biological disintegrated sludge component characteristic

Sample	Specific energy, [kWh/kg d.s.]	COD o [mg O_2/L]	COD f , [mg O_2/L]	d.s. (dry substance), [%]	v. s. (volatile substance), [% x d.s.]
M	0	19360	352	3.18	41.28
F1	0.45	19360	352	3.20	40.85
F2	2.71	20240	1408	3.17	41.23
F3	5.41	22000	1936	3.15	40.48

M - anaerobic fermented sludge sample with biologically thickened sludge component
FI, F2, F3 - anaerobic fermented sludge samples with ultrasonic disintegrated thickened biological sludge component.

Ultrasonic disintegration over the pharmaceutical compounds concentration is presented in Figure 1.

Ketoprofen was the only pharmaceutical compounds found in large concentrations and that exceeds the quantification limit in all the samples. As it can be observed, the concentration of these compounds decreases as the specific disintegration energy increases.

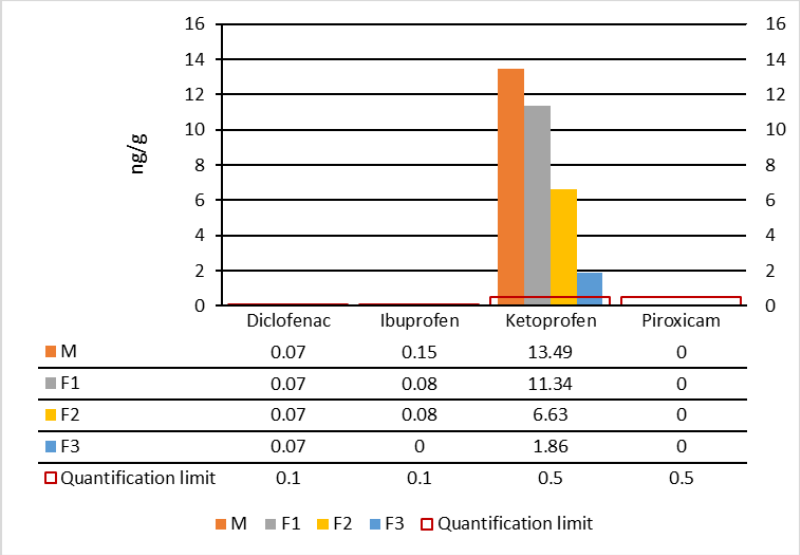

Figure 1. Pharmaceutical compounds concentrations variation in the treated sludge samples

As far as the determined hormone concentrations, estrone was the only hormone that was found beyond the limit of quantification, as it can be seen in Figure 2.

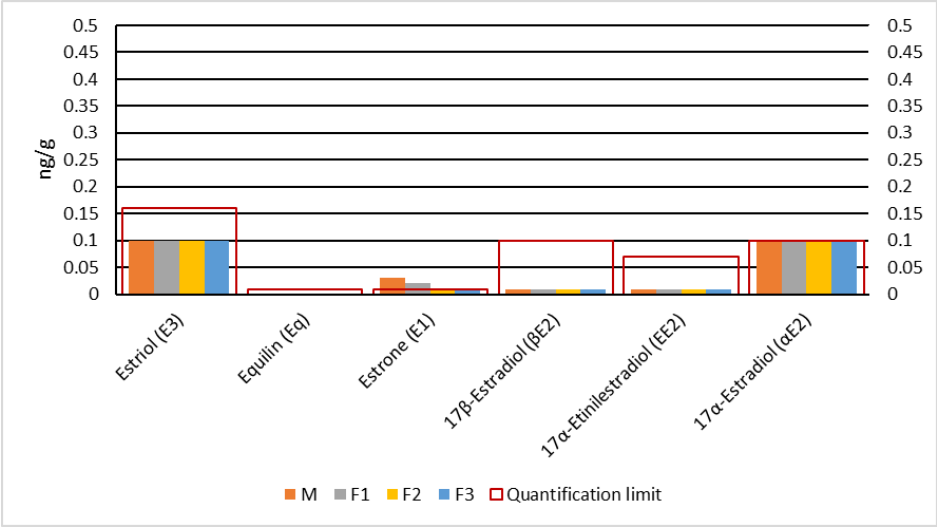

Figure 2. Hormones concentrations variation in the treated sludge samples

This hormone concentration slightly decreases as a response of ultrasonic digestion, without showing signs of being sensitive to the specific disintegration energy. Therefore, the same remanent concentration (0.012 ng/l) was found in both sample F2 (specific energy 2.71 kWh/kg d.s.) and F3 (specific energy 5.41 kWh/kg d.s.).

Similar to the pharmaceutical compounds, PAH concentrations generally exceed the quantification limit (Figure 3), but show sensitivity towards the applied disintegration energy, decreasing as the specific disintegration energy increases. Therefore initial phenanthrene concentration (62.4 µg/kg) decreased at a specific energy of 5.41 kWh/kg d.s (F3 sample) below the quantification limit (< 3 µg/kg). Floranthene presented sensitivity at applied specific energies ≥ 2.71 kWh/kg d.s, the remanent concentration being below the quantification limit. Initial chrysene concentration exceeded the quantification limit aprox. 7 times. Final concentration 4.2 µg chrysene/kg was obtained at applied specific energies ≥ 2.71 kWh/kg d.s.

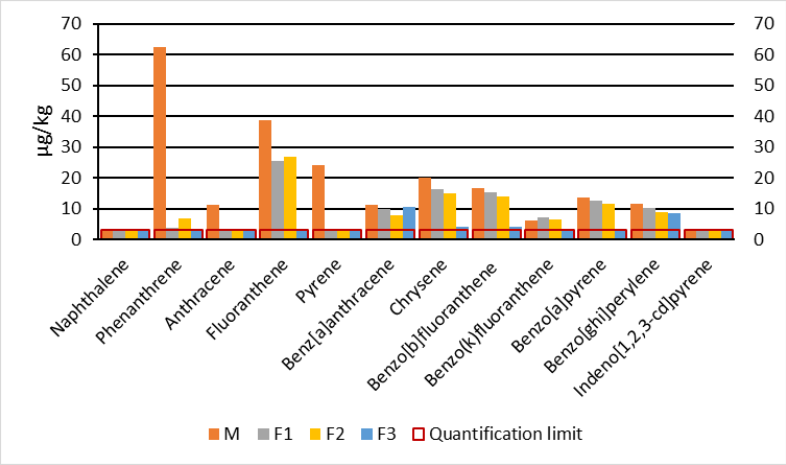

Figure 3. PAH concentrations variation in the treated sludge samples

3.2. Ultrasonic + Electrokinetic sludge disintegration

In order to evaluate the effects of both ultrasonic disintegration and electrokinetic disintegration of the thickened biological sludge over the same compounds (pharmaceutical compounds, hormones and PAH concentrations), samples of anaerobic fermented sludge were collected and analyzed. The same reaction mixture component (thickened biological sludge: thickened primary sludge: fermented sludge / inoculum in a 3: 1: 2 ratio) was used. The anaerobic fermented sludge characteristics are presented in Table 2.

Table 2. Anaerobic fermented sludge with ultrasonic biological disintegrated sludge component characteristic

Sample	Electrokinetic disintegration energy, [kWh/kg d.s.]	Ultrasonic disintegration energy [kWh/kg d.s.]	COD o, [mg O_2/L]	COD f, [mg O_2/L]	d.s., [%]	v.s., [% x d.s.]
M	0	0	22000	924	2.18	55.87
F1	0.0044	3.5	19360	1144	2.09	55.05
F2	0.0132	3.46	21120	1540	2.05	55.20
F3	0.0528	3.77	24640	1436	2.01	55.32

M - anaerobic fermented sludge sample with biologically thickened sludge component
FI, F2, F3 - anaerobic fermented sludge samples with ultrasonic and electokinetc disintegrated thickened biological sludge component.

Ultrasonic + Electrokinetic disintegration of the studied pharmaceutical compounds (analgesic class)-concentration in presented in Figure 4. Large concentrations of ketoprofen were identified. The concentration decreased from an initial 7.61 ng/g to 5.94 ng/g in F3 (electrokinetic disintegration energy 0.0528 kWh/kg d.s. + ultrasonic disintegration energy 3.77 kWh/kg d.s.). Ibuprofen concentration (0.51 ng/g initial) were reduced to half (0.25 ng/g) under the same ultrasonic and electrokinetic disintegration energy (sludge sample F 3 - electrokinetic disintegration energy 0.0528 kWh/kg d.s. + ultrasonic disintegration energy 3.77 kWh/kg d.s.)

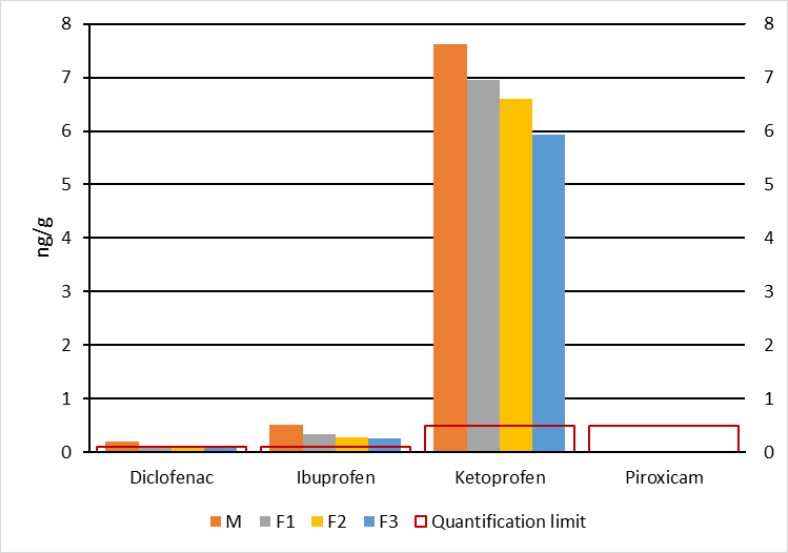

Figure 4. Pharmaceutical compounds concentrations variation in the treated sludge samples

The estrone concentrations (Figure 5) decreased from an initial 0.06 ng/g to 0.01 ng/g (quantification limit) in the same F3 sludge (electrokinetic disintegration energy 0.0528 kWh/kg d.s. + ultrasonic disintegration energy 3.77 kWh/kg d.s.). The other hormones identified were under the quantification limit.

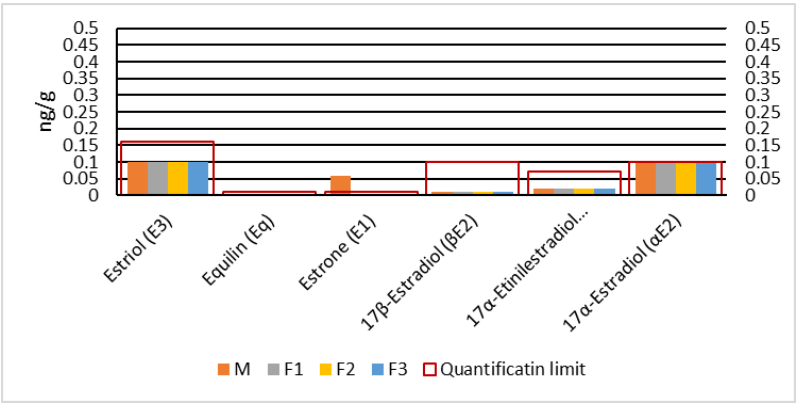

Figure 5. Hormones concentrations variation in the treated sludge samples

PAH concentrations, presented in Figure 3, show sensitivity towards the applied disintegration energy, decreasing as the specific disintegration energy increases, with the exception of fluoranthene. After the combined disintegration process was applied, an electrokinetic energy of 0.0528 kWh/kg d.s. + ultrasonic disintegration energy 3.77 kWh/kg d.s., initial concentration 13.4 µg/kg decreased to 9.7 µg/kg, pointing out fluoranthene low sensitivity to the applied ultrasonic + electrokinetic disintegration.

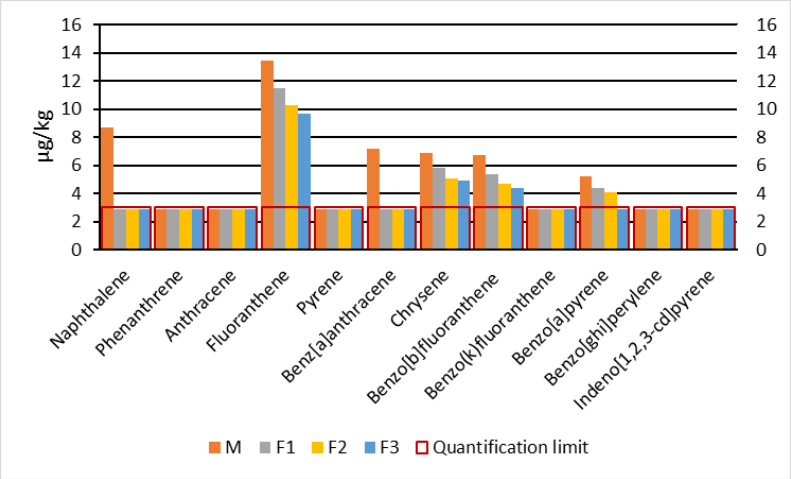

Figure 6. PAH concentrations variation in the treated sludge samples

As it can be observed, the applied ultrasonic + electrokinetic sludge disintegration,can reduce the initial concentration of the studied emerging pollutants. The concentration of these compounds show a tendency to decrease at an increased electrokinetic energies (≤ 0.0528 kWh/kg d.s.) and a relative constant ultrasonic disintegration energy (~ 3.5 kWh/kg d.s.).

3.2. Ultrasonic + Electrokinetic + Microbubble generation sludge disintegration

The last experiments focused on using a combination of the three disintegration process: ultrasonic, electrokintic and microbubble generation. The anaerobic fermented sludge characteristics subjected to this type of combined process are presented in % Table 3.

Table 3. Anaerobic fermented sludge with ultrasonic biological disintegrated sludge component characteristic

Sample	Electrokinetic disintegration energy, [kWh/kg d.s.]	Ultrasonic disintegration energy [kWh/kg d.s.]	COD o, [mg O_2/L]	COD f, [mg O_2/L]	d.s., [%]	v.s., [% x d.s.]
M	0	0	18480	590	2.34	56.93
F1	0.033	0.47	18480	669	2.06	56.26
F2	0.066	0.94	20240	686	2.00	56.01
F3	0.066	3.58	21120	748	2.06	57.87

M - anaerobic fermented sludge sample with biologically thickened sludge component
FI, F2, F3 - anaerobic fermented sludge samples with ultrasonic, electokinetc and microbubble disintegrated thickened biological sludge component.

In contrast to the previously experiments, the dominant pharmaceutical compound was diclofenac (Figure 7) with an initial concentration of 3.23 ng/g. After the combined disintegration process was applied, an electrokinetic energy of 0.066 kWh/kg d.s. and 3.58 kWh/kg d.s. ultrasonic energy, the lowest remanent concentration registered was 0.64 ng/g (F3 sludge sample). Slightly large concentrations of ketoprofen (1.42 ng/g initial) and ibuprofen (0.51 ng/g) were also identified. The lower concentrations of these two pharmaceutical compounds (0.9 ng/g – remanent ketoprofen concentration and 0.25 ng/g – remanent ibuprofen concentration) were also registered in F3 sludge sample.

Estrone seems to be the only identified hormone that exceeds the quantification limit (Figure 8) and was present in all the experiments. Its initial concentration 0.09 ng/g was reduced to half (0.04 ng/g) after the applied ultrasonic + electrokinetic + microbubble generation sludge disintegration process.

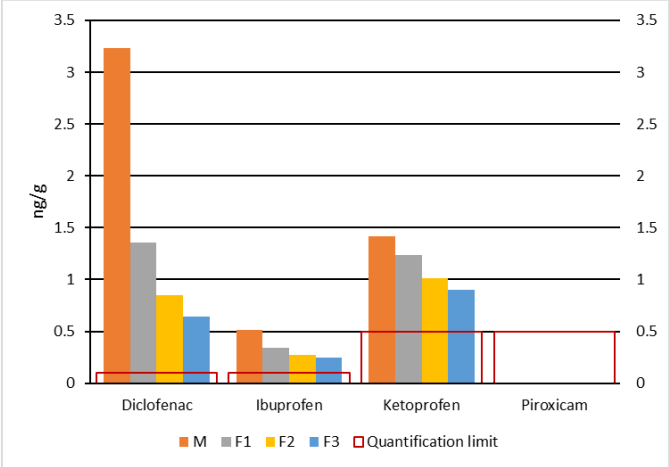

Figure 7. Pharmaceutical compounds concentrations variation in the treated sludge samples

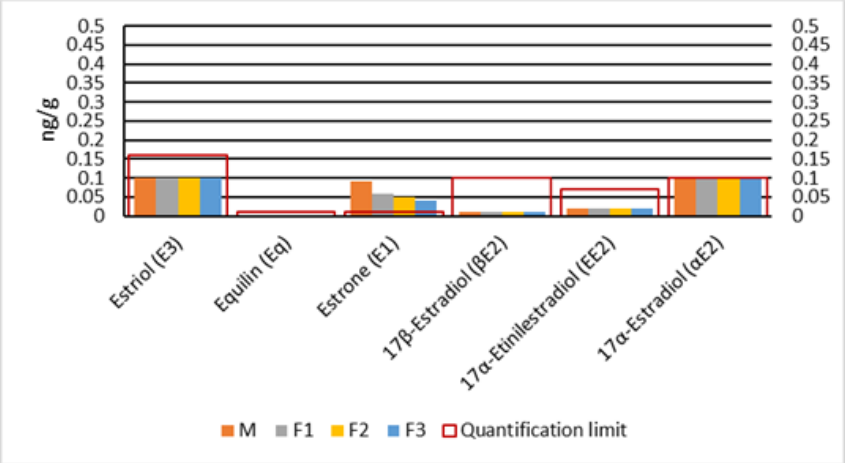

Figure 8. Hormones concentrations variation in the treated sludge samples

As observed previously and similar to the other two experiments described, the PAH concentrations, shown in Figure 9, with values above LOQ, showed sensitivity to the applied disintegration energy, they decreased as the specific disintegration energy increased. Phenanthrene, fluoranthene, pyrene and benzo[ghi]perylene concentrations were reduced to approx. half after applying ultrasonic + electrokinetic + microbubble generation sludge disintegration process.

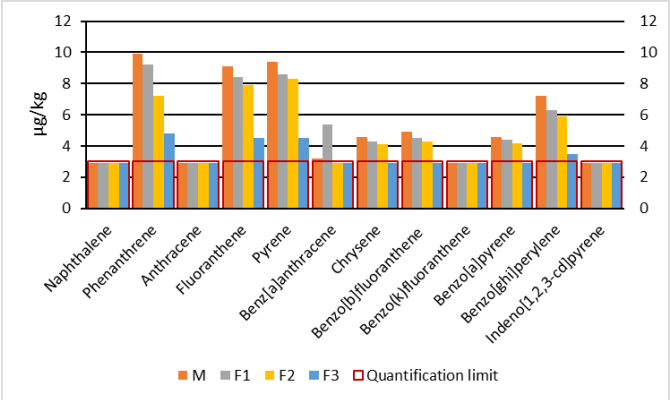

Figure 9. The PAH concentrations variation in the treated sludge samples

Ultrasonic + electrokinetic + microbubble generation sludge disintegration process can reduce the studied emerging pollutants concentrations due to in particular ultrasonic disintegration energy increase (0 – 3.58 kWh/kg d.s.).

4. Conclusions

The main pharmaceutical compounds predominant in all of the experiments were ketoprofen, ibuprofen and diclofenac in various concentrations. The only identified hormone in all of the cases was estrone. All of the targeted compounds (pharmaceutical, hormones and PAH) were sensitive to the applied disintegration processes, the experimental results showing the decrease of their concentration correlated with the increase of the disintegration energy.

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