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RESEARCH ARTICLE

BIOLOGICAL EFFECTS OF ENVIRONMENTALLY RELEVANT CONCENTRATIONS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUG (DICLOFENAC) ON FRESHWATER SHRIMP, *GAMMARUS PULEX*

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Abstract

All over the world pharmaceuticals are widely used to treat human diseases and to maintain healthy life but the aquatic environment is awash with these drugs leading to environmental health concern. Pharmaceutical exposure leads to toxicity in many non-target organisms, such as freshwater shrimp, *Gammarus pulex*. In this study, the biological effects of diclofenac on growth, feeding and mortality of aquatic macro-invertebrate shrimp (*Gammarus pulex*) was investigated in a laboratory experiment. Test concentrations were selected to mimic environmental detection levels reported for UK rivers in the literature. Seventy-five (75) *G. pulex* assigned random among the five experimental groups. were exposed to environmentally relevant concentrations of diclofenac (202.2 ngL^{-1}, 1596.45 ngL^{-1} and 2,990.7 ngL^{-1}) for four (4) weeks. There was decrease in mass and the growth rate decreased ($p<0.001$) significantly compared to controls. Also, the feeding rates in the controls were higher hence, the amount of feed materials consumed were dose dependant and significantly influenced by diclofenac ($p < 0.001$).Mean mortality was more than 80 % in the high treatment in the 3rd week of the experiment and there were statistically significant differences between the treatments and controls ($p < 0.05$). These results showed that exposure of *G. pulex* to environmentally relevant concentrations of diclofenac have significantly affected feeding activity, impacted growth and increased mortality, suggesting that prolonged exposure, use of sensitive points (behavioural signs) and use of susceptible test species (*G. pulex*) are more useful for assessing sublethal impacts of contaminants and are sensitive indicators of toxicity in benthic macroinvertebrates animals. Hence, these tools are useful in the aquatic environmental risk assessment of drugs.

Introduction:-

Pharmaceuticals are developed and used for specific biological effects, being administered for human and animal health care, and livestock farming. In 2017, the pharmaceutical industry sold nearly $1,105 billion worth of drugs

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globally. With population that is fast aging and technology aided cheap manufacturing processes, production of pharmaceuticals is expected to grow between 4 to 7 percent annually. With this heavy dependant on medicine, pharmaceuticals are rapidly being discharged into the aquatic environment and because of their physicochemical and biological properties, there are concerns about the potential for their impacts to non-target aquatic species (Park, 2006; Ericson et al. 2010; Mehinto et al. 2010; Cooper et al. 2008; Tim Aus Der Beek et al., 2016). Exposure of non-target species to pharmaceuticals can have severe implications. As such there have been greater efforts to identify the modes of action of these chemicals and their potential effects on aquatic environments and the organisms that are part of them. In addition, there has been increasing effort to quantify the extent to which these pharmaceuticals are present in aquatic environments and their routes of entry (Santos et al. 2010; Bendz et al. 2005). It has been established that the main routes of entry for pharmaceuticals into aquatic environments is through the effluent from sewage treatment plants (STPs) (Ternes 1998; Hughes et al., 2013). Other sources are excretion through urine, accidental spillage from manufacturing facilities, improper disposal and landfill leachates.

Diclofenac sodium salt (Figure 1) is a non-steroidal anti-inflammatory drug (NSAID) with antipyretic and analgesic actions. Diclofenac is widely used all over the world in the treatment of rheumatoid arthritis (Park et al. 2006) and also used in livestock treatment as an analgesic because of similar therapeutic properties exhibited in other mammals (Taggart et al. 2007) in spite of its risk of cardiovascular disorder. It is found on 74 countries list of essential drugs. Moreover, analyses of sales and prescription data from 15 high, medium and low-income countries revealed that diclofenac was the most commonly used NSAID, hence the reason their distribution in the aquatic environment is global. There is no record for sales figure in Nigeria for diclofenac, however, in United Kingdom, the diclofenac sales figure rose to £10.8 million in 2016 (Larsson et al, 2016) and in 2015 the drug was removed as an over-the-counter drug by the Medicines and Healthcare Products Regulatory Agency (MHRA) of UK. The use of diclofenac in cattle caused a considerable decline in vultures in India and Pakistan. The Gyps genus of vulture was surprisingly sensitive to residues of diclofenac in dead cows on which they fed, leading to acute renal failure and visceral gout (Oaks et al 2004). Diclofenac has since been withdrawn as a veterinary medicine (Kumar, 2006). However, it is still used widely as an analgesic in human medicine; it is persistent through sewage treatment and is regularly detected in effluent and surface waters around the world (Hoeger et al., 2005, Taggari et al., 2007).

Many of these compounds are not completely absorbed or metabolised by the human body and are excreted either having only been partially broken down or completely unchanged (Heberer 2002). These excreted products eventually find their way into municipal STPs and in many cases, are discharged into aquatic environments. Many pharmaceutical agents may pass through STPs without being completely broken down and exist in aquatic environments in their unchanged, active form. In addition, these compounds may not be readily biodegradable in aquatic environments and so may persist for extended periods of time (Hartmann et al. 2008; Heberer 2002; Achilleos et al. 2010). Studies carried out in Europe and North America found as many as 80 compounds of pharmaceutical origin present in aquatic environments. These compounds were isolated both in effluent from STPs and in surface waters downstream from these STPs (Heberer 2002). This suggests that these compounds are not exclusive to areas directly adjacent to the point of entry but may instead be ubiquitous in the world's aquatic environments

G. pulex was chosen as a test animal because they play an important role in the food chain and therefore their loss or reduced abundance has the potential for widespread ramifications to aquatic ecosystem. They are also bio indicators of the stream health, generally abundant, easy to sample and most likely to be affected by pollution because they have little mobility. They play an important role in the decomposition of coarse particulate organic matter, an important prey for many fish and non-piscean predators (McCahon et al., 1991), they are generally classified as an omnivorous shredder (Vanderford, 2006). *G. pulex* has been successfully used in a variety of toxicity tests, including feeding activity (Taylor et al., 1993), precopula separation (Pascoe et al., 1995), scope for growth (Maltby et al., 1990), in situ tests (Crane and Maltby, 1991), and behaviour (e.g. Graca et al., 1993b).

The current work investigated the ecological effects of prolong low-level exposure of *G. pulex* to diclofenac at environmentally relevant concentrations on growth, feeding and mortality with the aim of broadening knowledge about the potential risk of such contaminant to aquatic ecosystems.

Materials and Methods:-

Materials:

DIC (CAS no.15307-79-6, purity > 98%, $C_{14}H_{10}Cl_2NNaO_2$) was purchased from Sigma-Aldrich, UK. High performance liquid chromatography (HPLC) grade methanol (CAS no.67-56-1, purity ≥99.9%) was purchased from Fisher Scientific UK. Deionised water was generated with Purite Select HP160/BP/IT deionizer. Chemical stock solution was prepared in methanol on a weight basis in 100 ml of 100 % methanol and stored at −20 °C, and the working solutions were diluted aliquots of the stock solutions. Glassware and vessels were disinfected then pre-rinsed with 100 % methanol and deionised water and left to dry in the fume cupboard prior to the experiments.

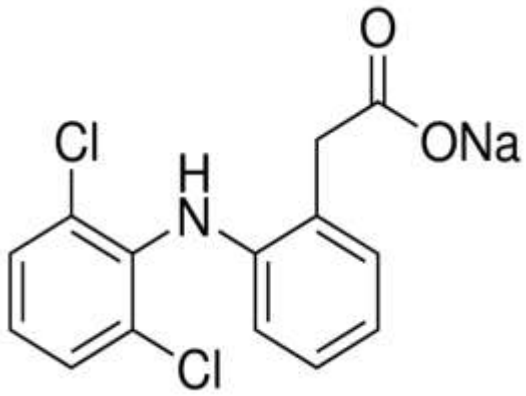

Figure 1:- Diagram of diclofenac sodium salt showing chemical composition, image from: (Sigma-Aldrich).

Methods:

The experiment was carried out in clear glass SS jar (500 mL) with face lined cap kept in incubators at a temperature of 12º C and 16:8 h light: dark regime. The animals were illuminated with a fluorescent light (with a specification for freshwater invertebrates), to simulate on a small scale the macroinvertebrates natural climatic conditions. The glow mimicked the thermal warmth and daytime illumination obtained from the sun radiation.

Each glass jar contained one *G. pulex* with 300 ml of pond water, which was assigned randomly, weighed individually at the start of the experiment and subsequently every week with Sartorius (model: Quintex 224-1s) balance. Exposure glass jars were arranged randomly to avoid the influence of potential gradients in the incubator, and all the *G. pulex* were assigned in the experimental chambers using random integer generator to avoid subjectivity of the experimenter. Water samples for chemical analyses of the compounds were collected every week and analysed. They were fed with 0.8 gm (wet weight) of standardised alder leaves. There were three treatments (LT, MT and HT), negative and solvent controls with 15 replicates of each treatment and 15 replicates of each control. The solvent control used in the experiment was tested for different responses of the physiological measurements compared to the negative control. No statistical difference was found between control treatments with and without solvent.

Test concentrations were selected to mimic environmental detection levels reported for UK rivers in the literature. The low treatment (LT) was UK mean measured environmental concentration of 202.2 ngL^{-1} (LT), 1596.45 ngL^{-1} medium treatment (MT) and 2,990.7 ngL^{-1} high treatment (HT) for diclofenac respectively (Hughes et al., 2013, Bound and Voulvoulis, 2006). The negative control contained no treatment and the solvent control contained 0.1 ml L^{-1} of methanol. The working solutions of LT, MT and HT were poured on a transparent silica glass beads and allowed to evaporate to dryness in the fume cupboard in other to avoid methanol toxicity, then the dried extracts were reconstituted/resuspended with 10 mL of pond water and washed into the beakers before *G. pulex* were introduced. After every use, the beads were rinsed with deionised water and ashed at 550° C for 4 hours. Separate beads were used for the different treatments and controls to prevent contamination.

In all seventy-five (75) male *G. pulex* were assigned at random among the five experimental groups. Exposures were static-renewal with 100 % water replacement every week with fresh concentrations of the pharmaceutical. The experiments were run for 4 (four) weeks. Response variables growth, mortality and physicochemical parameters were measured weekly and feed consumed measured at the end of the experiment.

Data analyses:
Data were organised using Excel (Microsoft, 2013) spreadsheet. Residuals of the data were checked for normal distribution (Kolmogorov and Smirnov method) and homogeneity of variance (Bartlett method). R (R Development Core Team, 2008) was used to analysed the data and create figures. Tukey's post-hoc tests were used to identify the means that differed. Change in *G. pulex* mass, physicochemical parameters and mass of feed materials (*Alnus glutinosa*) loss from week 1 to week 4 were tested using generalised linear model and Chi-square. Mortality were analysed using one-way ANOVA where assumptions of normality and homogeneity were met followed by a Tukey's post-hoc test to compare the treatment means with the respective controls.

Results:-

Growth:
When the experiment was initiated the mean mass of *G. pulex* was 21.84 ± 3.06 mg and no statistically significant difference (ANOVA: $F_{4, 70} = 0.42$, $p = 0.79$) were recorded between treatment groups and the control groups. In the experimental period *G. pulex* increased in mass in the control groups (NCTR: 24.33 ± 4.05 mg & SCTR: 23.33 ± 4.70 mg) and there were decreased mass in the treatment groups (LT: 21.63 ± 3.81 mg, MT: 18.17 ± 1.47 mg and HT: 14.00 ± 2.83 mg). However, mass decreased was more pronounced in the high dose treatment than the other treatments. At the end of the experiment statistically significant difference were found between the treatment groups and the control groups (ANOVA: $F_{4,41} = 9.75$, $p < 0.001$) figure 2.

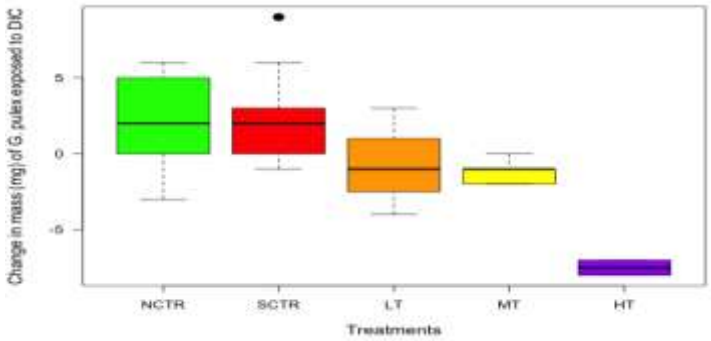

Figure 2:- Boxplots displaying change in mass of *G. pulex* exposed to environmental relevant concentrations of diclofenac after a 4 week static renewal experiments. Negative control (NCTR), solvent control (SCTR), low treatment (LT), medium treatment (MT) and high treatment (HT). The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There was outlier.

Feeding:
There were statistically significant differences in the mass of feed materials consumed between controls and treatments (ANOVA: $F_{4,70} = 42.19$, $p < 0.001$). The feeding rates in the controls were higher than the treatments. Even between the treatments group, the amount of feed materials consumed were dose dependant and significantly influenced by DIC (Figure 3).

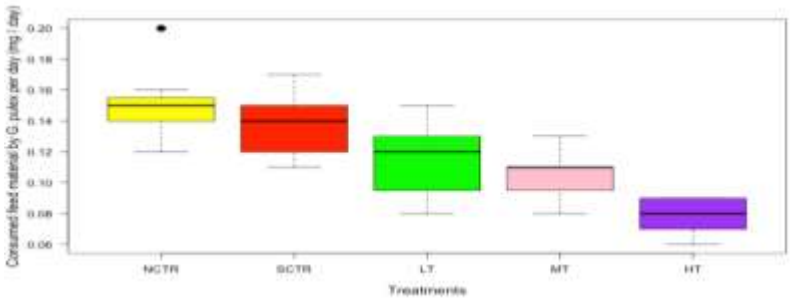

Figure 3: Boxplots displaying change in feed materials of *G. pulex* exposed to environmental relevant concentrations of diclofenac after a 4 week static renewal experiments. Negative control (NCTR), solvent control (SCTR), low treatment (LT),

medium treatment (MT) and high treatment (HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents 3rd quartiles (75th percentile), top whisker represents 4th quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There was outlier.

Mortality:

In the first week of the experiment there were no mortality recorded in all the treatments (LT, MT, HT) and the controls (NCTR, SCTR). The treatments started showing considerable increase in mortality from week two compared to the controls (figure 4). Mean mortality was more than 80 % in the high treatment in the 3rd week of the experiment, 60 % in the medium treatment, more than 40 % in the low treatment while in the controls there were no mortality. There were statistically significant differences between the treatments and controls (GLM: χ^2 (4) = 5502.3, $p < 0.05$)

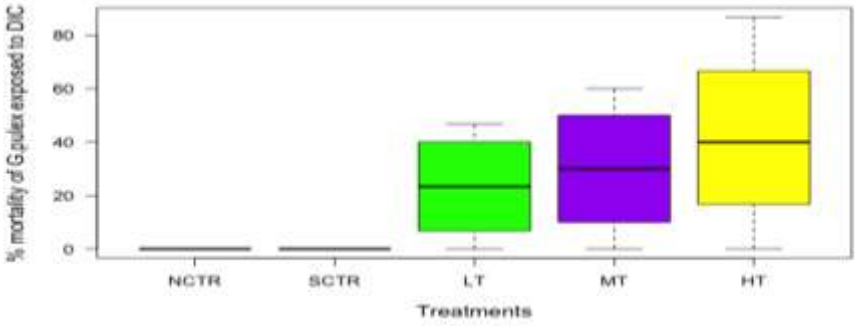

Figure 4:- Boxplots displaying % mortality of *G. pulex* exposed to environmental relevant concentrations of diclofenac after a 4 week static renewal experiments. Negative control (NCTR), solvent control (SCTR), low treatment (LT), medium treatment (MT) and high treatment (HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents 3rd quartiles (75th percentile), top whisker represents 4th quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There were no outliers.

Discussion:-

The objective of this study was to investigate the effects of environmentally relevant concentrations of diclofenac on growth, feeding and mortality of *G. pulex*. This study showed that growth and feeding behaviour of *G. pulex* were affected when exposed to environmentally relevant concentrations (202.20 ng L^{-1}, 1596.45 ng L^{-1} and 2990.70 ng L^{-1}) of diclofenac. There was growth retardation, *G. pulex* were not feeding, and mortality increased in a dose-dependent manner. These results are similar to effects on aquatic invertebrates reported by previous studies. For example, in a study conducted by De Lange et al. (2006) there was a decrease in the feeding behaviour of *G. pulex* when exposed to low concentrations (1-100 ng L^{-1}) of diclofenac and, in the same study, feeding rate of *G. pulex* when exposed to 10 ng L^{-1} of carbamazepine for one and half hours decreased by 45 % when compared to the controls.

This study was also in agreement with Fotedar and Evans (2011) in which *C. maenas* was exposed to environmentally relevant concentrations of diclofenac; it significantly reduced feeding and ultrastructural change was observed in the gill lamellae of the exposed crustacean. Also, when fingerlings of rainbow trout were exposed to 0.0071 mg L^{-1} of diclofenac there was modification in fish behaviour and abnormal swimming behaviour was reported (Orvos et al., 2002; Oliveira et al., 2009). Observations from this study are in agreement with some previous studies of aquatic organisms in which Quinn et al. (2014) demonstrated that exposure to carbamazepine at 50 mg L^{-1} for 96 hr significantly reduced feeding activity in *Hydra attenuata* and exposure to 10 mg L^{-1} diclofenac for 96 h significantly reduces the time for prey ingestion. However, this result is not in agreement with that of Richard et al. (2015), who reported that food intake increased in Wistar rats (though vertebrate animal) exposed to 2.5 mg kg^{-1} of diclofenac for 10 days. The observed difference in effects between *G. pulex* and Wistar rat exposed to diclofenac may be due to dose and species differences. Decrease in feed intake and growth in *G. pulex* are interrelated (Schmidt et al., 2011) and may have broad effects on growth, reproduction and population success. The reduction in feeding activity of *G. pulex* could links the concurrent decrease in growth rate. De Lange et al. (2009) hypothesize that reduced feeding in *G. pulex* exposed to carbamazepine or diclofenac may interfere with growth. We equally hypothesize that the observed decline in action by diclofenac can inhibit *G. pulex* behaviour, for example,

avoidance of predator (locomotion) and feeding behaviour. Reduced feeding will unavoidably cause reduced intake of energy, which can have far-reaching consequences on growth and reproduction. Change in predator avoidance behaviour will distort the predator–prey balance in the ecosystem. This may have a short-term positive impact for the predator (i.e. increased prey consumption), however, long-term adverse effects may be observed when the prey source is overexploited. This observed reduction in activity of *G. pulex* in response to low levels of diclofenac is in accordance to its pharmacological purpose in humans (Fent et al., 2006). Although the mechanism of diclofenac toxicity is not fully understood but there is some evidence from previous study by Mastrangelo et al. (2014) that showed diclofenac hindered serotonin-induced reproducible levels in pig ureter. Inhibition of feeding behaviour by diclofenac may be related to the functions of serotonin (5-HT) as a neurotransmitter and also coordinate behaviours including feeding activities (Fent et al., 2010; Barton et al., 2013). Nephrotoxicity of diclofenac is thought to be mainly due to the inhibition of prostaglandin synthesis and subsequent changes in prostaglandin regulated mechanisms, such as vessel tone, vascular permeability and ion regulation (Sanchez et al., 2002). Previous studies were able to demonstrate that diclofenac can inhibit cyclooxygenase activity and accordingly, synthesis of prostaglandin E2 in brown trout head, kidney, macrophages in vitro, thus demonstrating the same mode of action as reported for mammalian species. The effects of non-steroidal anti-inflammatory drugs on prostaglandin synthesis in humans are known to result in hyperkalaemia and hyponatraemia. Hickey et al. (2001) suppose that oxidative damage and subsequent necrosis and possibly apoptotic cell death also play an important role in diclofenac-induced nephrotoxicity. In mammalians, prostaglandins are known to be principal regulators of blood circulation and ion concentrations in kidney and gills in fish. It is feasible to assume that in aquatic invertebrates' prostaglandins may also display similar mechanistic roles and biological mechanisms. Consequently, *G. pulex* exposed chronically to low levels of diclofenac could suffer adverse effects associated with the inhibition of COX and PGE2 synthesis. Diclofenac is a small molecule, with a log Kow of 0.7 (sodium-diclofenac) and low lipophilicity. It may therefore easily pass through cell membranes.

Conclusion:-

These results showed that exposure of *G. pulex* to environmentally relevant concentrations of diclofenac have significantly affected feeding activity, impacted growth and increased mortality, suggesting that prolonged exposure, use of sensitive points (behavioural signs) and use of susceptible test species (*G. pulex*) are more useful for assessing sublethal impacts of contaminants and are sensitive indicators of toxicity in benthic macroinvertebrates animals. Hence, these tools are useful in the aquatic environmental risk assessment of drugs. Investigation needs to be done to find the effects of pharmaceuticals in Nigeria/Africa waterbodies.

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References:-

1. Achilleos, A., Hapeshi, E., Xekoukoulotakis, P., Mantzavinos, D., & Fatta-Kassinos, D. (2010). Factors affecting diclofenac decomposition in water by UV-A/TiO2 photocatalysis. Chemical Engineering Journal , 161, 53-59.
2. Bachhav, Y. G., Heinrich, A., & Kalia, Y. N. (2011). Using laser microporation to improve transdermal delivery of diclofenac:. European Journal of Pharmaceutics and Biopharmaceutics , 78, 408-414.
3. Barloche F., Kendrick B. (1973b) Fungi in diet of Gammarus pseudolimnaeus (Amphipoda).
4. Oikos 24:295-300. DOT: 10.2307/3543888
5. Bentley C.R., Hurd H. (1993) Pomphorhynchus laevis (Acanthocephalan) - Elevation of hemolymph protein concentrations in the intermediate host, Gammarus pulex (Crustacea,Amphipoda). Parasitology 107:193-198.
6. Bound, J.P., Voulvoulis, N. (2006). Predicted and measured concentrations for selected
7. pharmaceuticals in UK rivers: implications for risk assessment. Water Res. 40 (15),
8. 2885-2892.
9. Cooper, E. R., Siewicki, T. C., & Phillips, K. (2008). Preliminary risk assessment database and risk ranking of pharmaceuticals in the environment. Science of the Total Environment , 398, 26-33.
10. Crane M., Maltby L. (1991) The lethal and sublethal responses of Gammarus pulex to stress
11. Sensitivity and sources of variation in an insitu bioassay. Environmental Toxicology and
12. Chemistry 10:1331-1339. DOT: 10.1897/1552- 8618(1991)10[1331:tlasro]2.0.co;2.

13. De Lange, H. J., Noordoven, W., Murk, A. J., Lurling, M., & Peeters, E. T. (2006). Behavioural responses of Gammarus pulex (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. Aquatic Toxicology , 78, 209-216.
14. Ericson, H., Thorsen, G., & Kumblad, L. (2010). Physiological effects of diclofenac, ibuprofen and propranolol on Baltic Sea blue mussels. Aquatic Toxicology , 99, 223-231.
15. Fotedar, S., & Evans, L. (2011). Health management during handling and live transport of crustaceans: A review. Journal of Invertebrate Pathology , 106, 143-152.
16. Graca M.A.S., Maltby L., Calow P. (1993b) Importance of fungi in the diet of Gammarus pulex
17. and Asellus aquaticus. 2. Effects on growth, reproduction and physiology. Oecologia 96:304-309. DOI: 10.1007/bf00317498.
18. Hartmann, J., Bartels, P., Mau, U., Witter, M., Tumpling, W. v., Hoffman, J., et al. (2008).
19. Degradation of the drug diclofenac in water by sonolysis in presence of catalysts.
20. Chemosphere , 70, 453-461.
21. Heberer, T. (2002). Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicology Letters , 131, 5-17.
22. Hughes, S.R., Kay, P., Brown, L.E. (2013). Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ. Sci. Technol., 47, (2) 661-677.
23. Larsson, E., al-Hamimi, S., Jonsson, J.A., (2014). Behaviour of nonsteroidal anti-inflammatory
24. drugs and eight of their metabolites during wastewater treatment studied by hollow fibre liquid phase microextraction and liquid chromatography-mass spectrometry. Sci. Total Environ. 485e486, 300-308.
25. Maltby L., Naylor C. (1990) Preliminary-observations of the ecological relevance of the Gammarus scope for growth assay - Effects of zinc on reproduction. Functional Ecology 4:393-397. DOI: 10.2307/2389601
26. McCahon C.P., Poulton M.J. (1991) Lethal and sublethal effects of acid, aluminium and lime
27. on Gammarus pulex during repeated simulated episodes in a welsh stream. Freshwater Biology 25:169-178. DOT: 10.1111/j.1365- 2427.1991.tb00482.x
28. Mehinto, A. C., Hill, E. M., & Tyler, C. R. (2010). Uptake and Biological Effects of Environmentally Relevant Concentrations of the Nonsteroidal Anti-inflammatory Pharmaceutical Diclofenac in Rainbow Trout (Oncorhynchus mykiss). Environmental Science and Technology , 44, 2176-2182.
29. Oliveira, F. R. A. ; Oliveira, F. A. ; Guimarães, I. P. ; Medeiros, J. F. ; Oliveira, M. K. T. ; Freitas, A. V. L. ; Medeiros, M. A., 2009. Emergency of seedlings of Moringa oleifera Lam irrigated with water of different levels of salinity. Biosci. J., 25 (5): 66–74
30. Park, J. Y., Pillinger, M. H., & Abramson, S. B. (2006). Prostaglandin E2 synthesis and secretion: The role of PGE2 synthases. Clinical Immunology , 119, 229-240.
31. Pascoe D., Kedwards T.J., Blockwell S.J., Taylor E.J. (1995) Gammarus pulex (L) feeding bioassays - Effects of parasitism. Bulletin of Environmental Contamination and Toxicology 55:629-632.
32. Sanchez, S., De La Lastra, C. A., Ortiz, P., Motilva, V., & Martin, M. J. (2002). Gastrointestinal Tolerability of Metamizol, Acetaminophen, and Diclofenac in Subchronic Treatment in Rats. Digestive Diseases and Sciences , 47 (No 12), 2791-2798.
33. Santos, L.H.M.L.M., Araujo, A.N., Fachini, A., Delerue-Matos, C., Montenegro, M.C.B.S.M., (2010). Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J. Hazard. Mater. 175 (1-3).
34. Schmidt, A., Endo, N., Rutledge, S.J., Vogel, R., Shinar, D. and Rodan, G.A. (1992) Identification of a new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acids. Molecular Endocrinology, 6(10), pp 1634-1641
35. Taggart, M. A., Cuthbert, R., Das, D., Sashikumar, C., Pain, D. J., Green, R. E., et al. (2007). Diclofenac disposition in Indian cow and goat with reference to Gyps vulture population declines. Environmental Pollution, 147, 60-65.
36. Taylor E.J., Jones D.P.W., Maund S.J., Pascoe D. (1993) A new method for measuring the feeding-activity of Gammarus pulex (L). Chemosphere 26:1375- 1381. DOT: 10.1016/0045-6535(93)90188-b.
37. Ternes, T. A. (1998). Occurrence of drugs in German sewage treatment plants and rivers. Water Research , 32 (No 11), 3245-3260.
38. Tim Aus Der Beek, Frank-Andreas Weber, Axel Bergmann, Silke Hickmann, Ina Ebert, Arne Hain and Anette Kuster., 2016. Pharmaceuticals in the environment-Global occurrence and perspectives. Environ Toxi and Chem. 35 (4) 823-835.
39. Vanderford, B. J.; Snyder, S. A. (2006). Analysis of pharmaceuticals in water by isotope
40. dilution liquid chromatography/tandem mass spectrometry. Environ. Sci. Technol. 40 (23), 7312-7320.