CHAPTER 8.3

IMPACT OF GROWING ANTIBIOTIC POLLUTION ON AQUATIC ANIMALS

Ignacio Cabezudo[1], Maximiliano Cledon[2,3*]

[1]Instituto de Procesos Biotecnológicos y Químicos (IPROBYQ), Universidad Nacional de Rosario, Suipacha 577, Rosario, Argentina.

[2]Institut national de la recherche scientifique, Centre Eau Terre Environnement, INRS-ETE, 490, Rue de la Couronne, Québec, QC G1K 9A92, Canada.

[3]Instituto de Investigaciones Marinas y Costeras (IIMyC-CONICET), Funes 3350, Mar del Plata 7600, Argentina.

[*]Corresponding author, E-mail: maximiliano.cledon@ete.inrs.ca

Abstract:

Anthropogenic actions increment the bulks of nutrients, organic matter and hazardous substances in water bodies. Therefore, they are important driving forces impoverishing the status of many freshwater reservoirs. Among them, novel 'emerging' contaminants, like pharmaceuticals can be also accounted. Antibiotics produce multiple effects on mussels, such as haemocyte low viability, phagocitosis, thiol abnormal levels and others; ibuprofen diminishes lysosomal membrane stability in mollusks; diclofenac produces oxidative imbalances in mussels; anticancer agents can affect mussels and sea star; fluoxetine affects mRNA down-regulation in mussels; estrogens increase DNA damage in mussels DNA damage; finally, lipid regulators can lower lysosomal membrane stability. Pharmaceuticals also possess an effect on vertebrates Diclofenac affects growth and lipid peroxidation in zebra fish; ibuprofen decreases prostaglandine concentration in fathead minnows; antifungals decrease fertility of male zebra fishes; antidepresants decrease hunting ability of fathead minnows; antibiotics can produce histological changes in gills and liver of mosquitofish; antihypertensives can affect the glucose level of rainbow trout. Current research is developing potential remediation tools to improve the situation using grape polyphenolic extracts as chelants of heavy metals; or even zero-valent iron nanoparticles prepared with polyphenol extracts to remediate pharmaceuticals.

1. Introduction: importance of water quality towards life

Water covers 71% of the Earth's surface and thus is vital for life. It is estimated that 96.5% of the water is in seas and oceans, 1.7% is groundwater, and 1.7% is fixed in ice floes in the polar circles. A large part of water exists in water bodies, and only a very small fraction (0.001%) is suspended in the air as vapours, clouds, falling as precipitation. Therefore, 98.8% of this is held as ice and groundwater and only 2.5% of the Earth's water is fresh water. The availability of secure water that can be drunk has improved in the last few decades all over the world. Nevertheless, approximately a large part of the population, one billion still do not have access to safe drinking water, whereas another 2500 million people do not have adequate sanitation [1]. The availability of water and its physical, chemical and biological properties have an impact on the health of ecosystems. In the same fashion, there is a basic human requirement for an adequate stock of clean, usable water. Water is becoming scarcer as the human population continues to grow and demand high quality water for domestic use and economic activities. Rainfall has become unpredictable due to climate change [2]. Moreover, anthropogenic actions increment the bulks of nutrients, organic matter and hazards substances in water bodies, and, therefore, important driving forces for the insufficient status of many European freshwater reservoirs, as well as for the deficient quality of the water supplied to the populations [3]. For this reasons, the variability of chemical parameters must be evaluated and integrated in operational monitoring programs with the purposes of detecting changes of water status, characterizing the present status of water bodies. The main parameters need to be described, which must be remediated to restore the wellbeing of water bodies and guarantee its optimal condition for aquatic life (and for populations), providing information, to the authorities of each state member [4].

Respecting anthropogenic sources of pollution agriculture, in superficial waters, the bulks of nitrogen and phosphorus are significantly influenced by anthropogenic inputs associated with land cover, land use and circumscribed sources [5]. Poor animal agricultural uses are a great source of pollution, including: over scraped grass; over and excessive application including untimely utilization of pesticides, digging over irrigated fields and application of fertilizers. On the other hand, urbanization also implies its own anthropogenic inputs, such as conversion of grasslands, grasslands, marshlands, croplands, forests and other cover types to residential, transportation, profitable and industrial uses, thereby augmenting the areas of impenetrable surfaces.

Yet priority pollutants are not the only ones to contribute part of the large total chemical pollution. There is also a diverse group of novel, not regulated contaminants that have massively spread in the last decades into the environment on an extensive basis and are considered to be ‘emerging’. These chemicals include pharmaceutical and personal care products, flame retardants, industrial preservants, disinfection side products and insect repellents. Among emerging aquatic pollutants, pharmaceuticals, i.e. drugs used to diagnose, cure, treat, or prevent disease, are a large group of chemicals used either by humans or by agribusiness to enhance the growth or health of livestock [6]. Although information concerning the total annual production and use of pharmaceuticals is generally fragmentary, it has been reported that pharmaceuticals, including prescription drugs, veterinary drugs and diagnostic agents, are produced in large quantities, thousands of tons per year, worldwide, with huge environmental side effects [7]. In this context, Cleuvers [8] reported that several kilotons of non-steroidal anti-inflammatory drugs (NSAIDs), a group of substances widely used to treat pain and inflammation, are produced yearly. In Italy, the annual consumption of prescribed drugs was estimated at 209.58 tonnes of amoxicillin, 22 tonnes of β-blockers, 7.6 tonnes of antilipidaemics, 1.9 tonnes of ibuprofen, 26.67 tonnes of antacids and 6.4 tonnes of diuretics [9]. In the United Kingdom, 2.56 tonnes of fluoxetine (an antidepressant) were consumed in 2000 [10], whereas annual consumption of antibiotics in China was roughly 180,000 tons [11]. Regarding veterinary medicine, Sarmah et al. afforded an exhaustive review on the use and environmental occurrence of veterinary pharmaceuticals worldwide. In aquaculture particularly, the intensive farming that has been developed throughout the world requires the application of many pharmaceuticals, mostly antibiotics that constitute a major source of contamination [12].

2. Main sources of anthropogenic contamination

In rural areas, sediments are the most usual agricultural water contamination, as loss of top soil that is washed from the ground and carried and deposited in lakes or streams nearby, altering water nature. Some other agents such as pesticides, fertilizers, and heavy metals that hold to the soil are also washed into water bodies [13]. These pollutants cause algal blooms and drain oxygen, threatening aquatic life. On the other hand, overgrazing by livestock leads to exposure of soil and augmented erosion. This can result in ecosystem regression, encouraging intrusion of undesired species, flood plain vegetation and fish habitats destruction [14]. More complex organic molecules, such as pesticides, fungicides, insecticides and herbicides are used to prevent rural pests. They may enter water due to direct application, run-off from the fields or atmospheric deposition. Water may become polluted with a wide range of contaminants due to the use of land for agriculture [15].

Urban areas are generally more polluted than agricultural ones due to industrialization, sewage discharge and other domestic activities [16]. Current channelization can lead to changes in the bulk and speed of the water flowing through the channel. Surface waters are prone to pollution, as they are used for wastewater disposal in most countries. Sewage discharge is one of the most important cause of bacteria present in human faeces [17]. The main origins of contamination in rural and urban areas are summarised in figure 1, contamination with pharmaceuticals will be described in more depth in the next section.

3. Input of pharmaceuticals in the aquatic environment

Pharmaceuticals are generally absorbed by humans or animals after intake and partially metabolized by their target organisms. However, main parts of the original substances often are excreted in forms that are not metabolized or as actives via depositions to be spilled into raw sewage discharge, which may or may not be treated [18]. Moreover, when even when treated, some pharmaceutical pollutants escape degradation in debris treatment plants and enter the environment. A simplified scheme of potential sources and pathways of pharmaceuticals in aquatic environments was reported by [19] similar to the one on figure 2.

The concentration of pharmaceuticals detected in the aquatic environment is not only influences by usage levels, but also by the degree of metabolism that occurs in the patients body, degradation rates in the waste water system and receiving waters [20]. For example, ibuprofen has a high elimination rate, generally >90%, and so is rapidly degraded [21], whereas, in contrast, carbamazepine has an elimination rate of only 4–8% [22]. The slow elimination speed for carbamazine can ethe cause of why it can be frequently found in wastewater effluent and rivers, even though it is not a ahigh consumption pharmaceutical.

In contribution to metabolic excretion, disposal by flushing of unused or expired medication and medicine-containing debris from manufacturing facilities can also contribute to environmental contamination [23]. As an example, a large proportion of prescribed medicines are not administered and are disposed of directly into waste waters; an estimated $1 billion of prescription drugs are discarded each year in the US, from hospitals, care facilities, and pharmacies [7].

For all of these reasons, their occurrence in water bodies is frequent; in a 2002 nationwide study of "emerging pollutants" in waters, the U.S. Geological Survey (USGS) tested pharmaceuticals and personal care products in several U.S. Locations. Even in remote areas, 139 rivers in 30 states and detected a wide range of biologically active compounds in nearly 80% of the cases [24]**.** Moreover, North American waterways were determined to contain traces of APIs from a wide spectrum of therapeutic classes.

4. Effects of pharmaceuticals on invertebrates

It has been suggested that many cases of adverse effects in health in a range of wildlife species are due to exposure to disruptersof the endocrine system, such as pharmaceuticals. For invertebrates, which represent 95% of animal species, information on the effects and mechanisms of action of medicines is scarce compared to that available in vertebrates, given the complexity of endocrine systems in different phyla [25]The causes of the causes of apparition of species with genital characteristics common to both sexes in aquatic invertebrates are still not clear and a few mechanisms could be involved: parasitism, pollution altering parasite function, and pollution directly causing the intersexuality seem to be the main factors raising the frequency of the phenomenon ([26]. The effect of different groups of pharmaceuticals on invertebrates are summarised in the next paragraphs, and in table 1.

Antibiotics

Among pharmaceutical medicines in the environment, antibiotics are particularly important because they comprise a wide range of substances commonly used in medical and veterinary utilisation, aquaculture and livestock husbandry. This wide use constitutes a decisive factor committing for their frequent detection in the aquatic environment. The separate and combined effects of environmentally relevant concentrations of antibiotics on *Elliptio complanata* mussel immune parameters were assesed. Overall, the authors observed that antibiotics (ciprofloxacin, erythromycin, novobiocin, oxytetracycline, sulfamethoxazole and trimethoprim), alone and as mixtures, can induce immunotoxic effects at environmentally relevant concentrations [27]. Moreover, the data revealed that the removal of fine particles and microorganisms from municipal effluents can alter the toxic nature of the effluent that is closely associated with the cumulative effects of antibiotics.

NSAIDs

A very widely used non-steroidal anti-inflammatory medicine, ibuprofen, evidenced mediated lysosomal membrane destabilisation in hemocytes from *R. philippinarum* clams. The authors stated that the grade of toxicity calculated suggested that environmental concentrations in the μg/L range can be extremely toxic for the lysosomal membrane stability of clams [28].

On the other hand, another series of studies investigated the effects of diclofenac, on the hemocytes of the zebra mussel *D. polymorpha*, It was determined that environmentally relevant concentrations of diclofenac promoted negligible cellular and genetic damage. Only a slight decrease in lysosomal membrane stability was observed at the end of exposure at the highest concentration tested [29].

Anticancer agents

At present, only few studies were investigated, one is the study on the cytotoxicity and genotoxicity of cyclophosphamide (CP) on hemocytes from the mussel *Mytilus edulis* and celomocytes from the sea star *Asterias rubens*. In mussels, no significant effects on neutral red retention (NRR) assay were recorded, whereas a significant micronuclei induction and DNA strand break event was registered. In sea stars, no significant differences in NRR were observed between CP-exposed animals and seawater controls [30]. Conversely, significant increases in micronuclei induction and DNA strand breaks were detected after 5 and 7 days of exposure to 32 and 56 mg CP/l.

Lipid regulators

The effects of lipid regulators bezafibrate and gemfibrozil on immunocytes of *Mytilus* spp, the results indicated that environmental concentrations of hypolipaemic medicines can alter mussel immune system. Both compounds caused lysosomal destabilisation and extracellular lysozyme liberation, with a 50 % effect at 0.1 nmol. Contrariwise, phagocytic activity augmented (+24 %) at the highest concentration tested [31].

Antidepressants

In a recent study, the use of antidepressant agents to evaluate cAMP/PKA modulation and ABCB mRNA expression were assessed in hemocytes from the mussel *Mytilus galloprovincialis.* It was proved that fluoxetine significantly reduced cAMP levels and PKA activity and promoted ABCB mRNA down-regulation. The authors stated that their study affords the first evidence for the cAMP/PKA-mediated regulation of ABCB mRNA expression in mussels (Table 1). Overall, these results proved that the impairment of transduction pathways promoted by fluoxetine may alter the ability of mussels to cope with stressful conditions in the environment [32].

Estrogens

In the last decades, increasing attention has been given to evaluating negative effects of estrogens in aquatic organisms. One of the most documented effects of estrogens is the induction of vitellogenins, precursors of the egg-yolk proteins, vitellins, which afford energy reserves for embryo development. However, it has been proved that estrogens can also alter hemocyte parameters in aquatic invertebrates. [33] recently evaluated the effects of 17β-estradiol (E_2) on oxidative parameters of *M. galloprovincialis* hemocytes. Results proved that exposure of hemocytes to 25 nM of E_2 for 30 min caused a significant increase in ROS production and, consequently, a significant increase of DNA damage, protein carbonylation and lipid peroxidation. Increases in mRNA levels of the antioxidant enzymes CAT, SOD and glutathione S-transferase were also recorded (Table 1).

5. Effects of pharmaceuticals on vertebrates

Most chemicals entering the environment, have biological effects that generally occur as an unintended consequence of their principal function. Critically, pharmaceuticals are designed to alter physiological function, thus, some pharmaceuticals designed to induce an effect in humans or livestock have a high contingency of being biologically active in wildlife species. Supporting this hypothesis, ortholog testing of human gene drug targets was assessed, finding *Danio rerio* had orthologs to 86% of the drug targets while only 61% were conserved in *Daphnia* and 35% in green alga. The predicted presence and absence of orthologs complies well with published experimental data on the likelihood for specific drug target interaction in various species. Furthermore, individual targets, especially enzymes, are well conserved suggesting that tests on evolutionarily distant organisms would be highly relevant for certain medicines [34]. Thus, as a drug is developed based on a specific biological activity in one animal group (principally mammals) and many of these systems targeted are conserved amongst vertebrates, this results suggest that they will target the same systems in fish [35]. Some examples of studies on vertebrates using compounds from different groups of drugs, the reports on pharmaceutical effects on invertebrates are summarised on table 2.

NSAIDs

On a recent study on diclofenac, *Danio rerio* was subchronically exposed to sublethal grades of the medicine. The growth, oxidative stress, and histopathological changes were asssed. Fish were exposed to the diclofenac environmental concentration frequently detected in the Czech rivers and a decrease in the fish growth caused by diclofenac was observed in the highest concentrations. On the other hand, the environmental concentration of diclofenac in Czech rivers did not have any effect on *Danio rerio* but it could have an influence on lipid peroxidation [36].

Regarding another NSAID of popular use, ibuprofen effects on *Pimephales promelas* (fathead minnows) were tested by Patel et al. for evidence of read-across between species. Ibuprofen, a non-selective inhibitor of prostaglandins and the cyclooxygenase (COX) enzyme, could mimic its primary effect in humans, on fish, at comparable plasma concentrations. Results showed that PGEM grades in fish exposed to 370 and 470 μg ibuprofen/L were compellingly reduced compared to control fish. These data afford evidence for the read-across hypothesis, but suggest establishing a direct dose-response is difficult, and would require significantly larger numbers of fish to overcome the inter-individual variation [37].

Antidepressant

Effects of fluoxetine, an antidepressant that acts as a selective serotonin reuptake inhibitor (SSRI) were studied on striped bass. One important conduct that could be modified is the ability to capture prey. It was hypothesized that below lethal concentrations of fluoxetine can lead to feeding conduct abnormalities in hybrid striped bass (*Morone saxatilis*×*M. chrysops*). Effetively, the burden of sublethal fluoxetine exposures on the ability of hybrid striped bass to capture fathead minnows (*Pimephales promelas*). Exposed fish exhibited a concentration and duration-reliant decrease in ability to capture prey. Thus, sublethal exposure to fluoxetine decreases the ability of hybrid striped bass to feed on other fish and that serotonin decrease in brain can be used as a biomarker of exposure and effect [38].

Azole antifungals

Clotrimazole is an azole fungicide used as a human medicine that is known to hinder cytochrome P450 enzymatic activities. Chronic exposure to clotrimazole on zebrafish testis function, measuring the liberation of androgen and the production of sperm was tested. Study combined (1) gene transcript levels determinations along the brain–pituitary–gonad axis, (2) the quantification of ketotestosterone in blood, and (3) histology of the testes, including morphometric analysis. The study evidenced that clotrimazole is able to alter testicular physiology and raised further concern about the burden of clotrimazole on reproduction [39].

Antihypertensive

The sub-lethal effects and tissue concentration of the human pharmaceutical atenolol on *Oncorhynchus mykiss* (rainbow trout) were assesed. The main findings where that atenolol is a highly prescribed antihypertensive pharmaceutical and a member of the group of β-blockers. In blood plasma, fish exposed to 1 $\mu g.L^{-1}$ atenolol exhibited reduced haemoglobin and higher lactate content in blood plasma, compared with the control. Exposure to atenolol at concentrations greater than or equal to 10 $\mu g.L^{-1}$ showed to significantly reduce both the haematocrit amount and the glucose concentration in the blood plasma. The histological changes indicate that atenolol has an effect on the vascular system, but atenolol was established to have very low bioconcentration factors [40]The activities of selected CYP450 enzymes were not altered by atenolol exposure.

Antibiotics

Tetracyclines are some of the most frequently detected antibiotics in the environmental matrices. The depiction of the potential ecotoxicological effects of tetracycline in the freshwater fish species *Gambusia holbrooki* was determined by measuring the histological changes in the gills and liver, changes in antioxidant defense as well as potential neurotoxicity. The obtained results suggest the existence of a cause-and-effect liaison between the exposure to tetracycline and histological alterations in gills [41].

Recently reports are covering more thoroughly the effects of pharmaceuticals on aquatic species. The "read-across hypothesis" stipulates that pharmaceuticals will exhibit similar biological effects across species (e.g. human and fish) if the molecular target has been conserved and the effective medicine concentrations are reached [37].

6. The potential of polyphenols as green remedial agents

Plant phenolic compounds account for 40% of organic carbon circulating in the biosphere. Phenolic compounds are defined chemically by the presence of at least one aromatic ring that has one or more hydroxyl substituents including their functional derivatives [42]. Polyphenols have so far been identified in many by-products, as food, agro-industrial and forest residues, that can be regarded as renewable sources. Since polyphenols largely occur in green residues, they could be used at a reasonable cost in the clean-up of contaminated environments.

Regarding the remediation of antibiotics, zero-valent iron nanoparticles (nZVIs) were prepared using extracts of grape marc, black tea and vine leaves (all rich sources of polyphenols). This ‘green’ method also enhances the stability of the particles through the covering action of the polyphenols in the extract [43]. Utilization of green nZVIs to the remediation of soils contaminated with ibuprofen, allowed elimination of the anti-inflammatory (about 60% of the initial amount) in water solutions. These results indicate that this remediation technique represents a good alternative to more aggressive, traditional methods.

Polyphenolic compounds present in natural materials are sorption sites of highest importance for binding of metal ions. Thus biosorption of metal ions occurs as a result of ion exchange or complex occurrence between metal ions and the functional hydroxyl groups on the surface of the biomaterial bearing polyphenols. Moreover, [44] suggested the use of aqueous polyphenolic extracts obtained from red grape (Vitis vinifera) seed as ‘sequestrant’ to form chelates with heavy metals or as heavy metal biosolubilizers. Some other applications explored where chelating heavy metals and promote in situ inactivation of heavy metal ions in waters [45]. In the last decade, adsorbents have been synthesized from commercial tannins and used to remove various metal ions in wastewater. [46] highlighted the metal elimination efficiency of some tannin biosorbents showing that Pb(II) sorption by quebracho tannin resin was possible.

7. Future perspectives

With a growing human population, and increasing and more prevalent use of man-made chemicals, it is probable that fish populations will face increasing toxicological challenges. Global climate change will also burden aquatic environments and alter chemical exposures due to changes in dilution regimes [47]. The data summarised in Tables 1 and 2 indicates that the burden of pharmaceuticals on aquatic environments needs to be investigated more thoroughly; a variety of medicines can markedly influence immune parameters of non-target species. Nevertheless, efforts should be directed at evaluating the effects of drug mixtures because animals are more realistically exposed to complex drug mixtures in their environments. Therefore, some fish are exposed to a complex matrix of synthetic mixtures, and thus, identifying associations of health outcomes with specific compounds in natural populations is extremely difficult. Fundamentally, protection of fish and other wildlife operates at the level of populations and not individuals, and thus, advanced consideration on chemical effects requires not only enhanced understanding on the biological effects promoted by pharmaceuticals and their mixtures, but also the adaptive abilities and mechanisms of wild fish populations [48].

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9. References

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Table 1: reports on different groups of antibiotics effects on invertebrates (+ augments, - diminishes, ± effect depends on the conditions)

Pharmaceutical	Species studied	Effect	Reference
Ciprofloxacin, erythromycin, novobiocin, oxytetracycline, sulfamethazole, trimethoprim (antibiotic)	Elliptio complanata	Haemocyte viability ± Phagocytosis ± Thiol levels ± Phagocytosis ± Lisozyme activity ± NO production ± COX activity ±	Gust et al., 2012
Ibuprofen (NSAID)	R. philippinarum	Lysosomal membrane stability -	Aguirre-Martínez et al., 2013
Diclofenac (NSAID)	D. polymorpha	DNA damage = Apoptosis = Micronuclei = Lysosomal membrane stability =	Parolini et al., 2011
Cyclophosphamide (anticancer agent)	Mytilus edulis Asterias rubens	Lysosomal membrane stability (M.e.) = Micronuclei (M.e.) + DNA damage (M.e.) + Lysosomal membrane stability (A.r.) = Micronuclei (A.r.) + DNA damage (A.r.) +	Canty et al., 2009
Bezafibrate Gemfibrozil (lipid regulators)	Mytilus spp.	Lysozyme release + NO levels + Phagocytosis – Lysosomal membrane stability -	Canesi et al., 2007
Fluoxetine (antidepressant)	Mytilus galloprovincialis	cAMP - PKA activity - ABCB mRNA -	Franzellitti and Fabbri, 2013
17β-estradiol (estrogen)	Mytilus galloprovincialis	ROS production + DNA damage + Protein carbonilation + Lipid peroxidation + CAT mRNA	Koutsogiannaki et al., 2014

Table 2: reports on different groups of antbiotics effects on vertebrate species (+ augments, - diminishes).

Pharmaceutical	Species studied	Effect	Reference
Diclofenac (NSAID)	Diverse species; *Danio rerio*	Fish growth - Lipid peroxidation -	Praskova et al., 2014
Ibuprofen (NSAID)	*Pimephales promelas*	Prostaglandin E metabolite -	Patel et al., 2016
Clotrimazol (azol antifungal)	*Danio rerio*	Testis function, (spermatogenesis and androgen release) -	Baudiffier et al., 2013
Tetracycline (antibiotic)	*Gambusia holbrooki*	Histological change in gills and liver + antioxidant defense - potential neurotoxicity +	Nunes et al., 2015
Atenolol (antihypertensive)	*Oncorhynchus mykiss*	Haematocrit value and glucose concentration in the blood plasma - Histological changes on the vascular system +	Steinbach et al., 2014
Fluoxetine (antidepressant)	*Morone saxatilis* × *M. chrysops*	Serotonine levels - Ability to catch prey -	Gaworecki and Klaine, 2008

Figure 1: general anthropogenic causes for water pollution

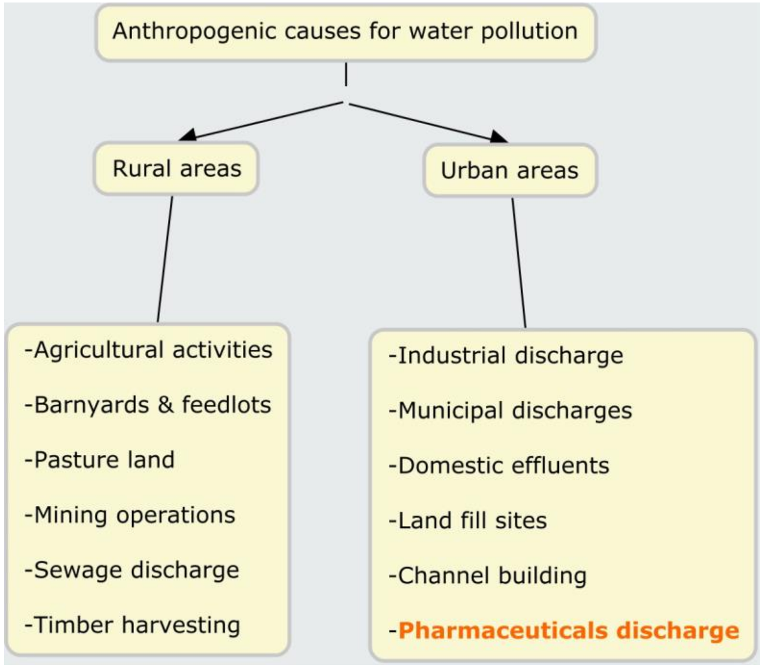

Figure 2. Main route of pharmaceuticals dissemination from anthropogenic sources to the environment.