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REVIEW

The Plankton Role in Pollutants Dynamics as a Tool for Ecotoxicological Studies

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Abstract:

Studies have evaluated the dynamics of pollutants at the base of the food chain to understand the contamination in the high levels of the trophic food webs (as fishes and humans). The base of the food chain, especially for those pollutants that biomagnifies, represents the beginning of the contamination of the trophic web. Those studies were possible from the last decades due to more precise analytical techniques. Studies have used mainly the zooplankton since this group has an important function in the transfer of matter and energy from phytoplankton, bacteria and materials (e.g.; inorganic sediments, organic matter in decomposition). These organisms can also transfer toxic substances to higher trophic levels since some pollutants can bioaccumulate in the zooplankton and biomagnify through food web. Therefore, understanding the role of plankton in the uptake of pollutants is fundamental to comprehend their concentrations in the food web.

Keywords: aquatic ecotoxicology; environmental toxicology; heavy metal; persistent organic pollutant; pollutants; trophic transfer

1. Initial Considerations

1.1 Planktonic community ecology

The planktonic community is composed by a variety of organisms, since unicellular until pluricellular ones, ranging from tiny bacteria, algae, and protists to microscopic and macroscopic animals (such as fish larvae). Planktonic organisms normally are classified by their composition, such as virioplankton (composed by virus), bacterioplankton (composed by prokaryotes bacteria or cyanobacteria), phytoplankton (composed by protists eukaryotes and microalgae), and zooplankton (composed by invertebrates, but also by fish larvae or eggs that can be sorted in the ichthyoplankton subgroup). The organisms also can be classified according to their size as picoplankton (0.2-2 μm), nanoplankton (2.1-20 μm), microplankton (20.1-200 μm), mesoplanktlon (201-2000 μm) and macroplankton (> 2000 μm) [1, 2].

This community is commonly described by the limited power of locomotion, being passively transported by the currents or water movements [1, 2]. These organisms have no abilities to overcoming the physical and chemical environmental barriers such as tide variance, waves, salinity and heat [3, 4]. However, the plankton community is able to do small displacements, mainly vertical migrations mostly due to the photoperiod, food resources and to avoid predators [5, 6].

1.1.1 Bacterioplankton

The bacteria have a fundamental role in the production (e.g.; cyanobacteria) and in the

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cycling of the organic matter. Thus, bacteria also recycle pollutants in the environment by returning them to abiotic environment, where they can be absorbed by the biota again [7–10].

1.1.2 Phytoplankton

Phytoplankton counts the most photosynthesizing organisms and, therefore, it is important for producing biomass, oxygen, and the initial bioaccumulation of several compounds, including pollutants. This group is also an indicator of environmental conditions, such as the community composition (e.g.; indicator species) or abundance (e.g.; blooms in eutrophic sites) [11].

1.1.3 Zooplankton

Zooplankton is characterized as a set of animals, microscopic or not, that inhabit the water column. They may be either whole life cycle in the water column (holoplankton) or be temporarily in this site (meroplankton), when the larvae is planktonic but the adult individual is nektonic or benthic [2, 3]. The zooplankton community is very diverse, ranging from unicellular to colonial individuals, such as heterotrophic protozoans and macroscopic animals, as some pyrosomes [3, 4, 12]. Ichthyoplankton, subgroup of zooplankton, is different in terms of recruitment and economic importance, and is represented by eggs and larvae of fish [2]. Zooplankton plays an essential role in the transfer of matter and energy from phytoplankton to other trophic levels [13]. Zooplanktonic organisms can incorporate several elements or compounds from phytoplankton or by the diffusion from the surrounding water [14].

2. Ecotoxicology of the Planktonic Community

Planktonic organisms are an interesting tool in ecotoxicology studies since they (1) are the main responsible for the transference of the pollutants from abiotic environment to biota; (2) can indicate site disturbance because they generally are the first organisms affected by pollutants; (3) have different trophic levels; and (4) recycle organic matter and pollutants concomitantly. Therefore, they are excellent tool to construct models about population and community effects or environmental cycle of pollutants [15]. The interactions among planktonic species promote the redistribution of pollutants to the organisms, influencing the dynamics of pollutants in the environment [16–18]. These interactions can increase or decrease the concentrations of pollutants in the aquatic food web [18–20].

2.1 Metabolization of pollutants

Bacteria can provide different processes of pollutants metabolization. Thus, the persistence or the bioaccumulation of pollutants can be increased or decreased depending on the product generated after metabolization. For example, the transformation of inorganic mercury in methylmercury (mercury methylation) mainly by sulfate-reducer bacteria results in a major bioaccumulation capacity of the mercury and increases the toxicity of this element [8, 21]. The methoxylation or the hydroxylation of polychlorinated biphenyls (PCBs) in hydroxylated polychlorinated biphenyls (OH-PCBs) or methoxylated polychlorinated biphenyls (MeO-PCBs) increase their persistence and bioaccumulation [7]. On the other hand, pollutants also can be degraded, decreasing their life cycle in the environment. Understanding this process, identifying which organisms have the capacity to degrade a pollutant is useful for bioremediation applications. For example, microbial degradation of hexachlorocyclohexanes was evidenced to occur in Arctic Ocean, that is very important to their removal from this environment [10].

2.2 Processes regarding on elements (biogeochemistry)

The planktonic organisms play important roles in different processes such as, the cycling of elements, the incorporation of energy and matter (or formation in the case of phytoplankton), and their transference to the food chain. In the recycling of bioelements, rather through ammonia and phosphate excretion, the planktonic organisms lead to rapid and direct exchange of nutrients between phytoplankton and zooplankton, according to the increase and the decrease of the biomass [22, 23]. Then, where plankton assume an important role of nutrients regeneration, a pollutant-caused reduction in plankton biomass could have a significant effect on natural ecosystem. Lipids richness is a very important thing in order to control the concentrations of pollutants, especially the lipophilic compounds. The quick dynamics in planktonic community and the large species variance is a key role in this process [22, 24, 25]. As plankton represents the initial level of many food chains, understanding the plankton ecology is very important in order to understand how the pollutants achieve, or not, different trophic levels.

3. Contaminants in the Environment

The environment has many natural or anthropogenic compounds that can bioaccumulate, biomagnify in the food chains (increasing their concentrations along the food chain) and/or be toxic to the organisms. The present study focus mainly on the anthropogenic organic pollutants and heavy metals (or trace elements).

3.1 Anthropogenic organic pollutants

The amount of chemicals used by humans for several purposes may be a threat for environmental health, including the organisms. Some anthropogenic compounds are pesticides (e.g.; chlorine and phosphate pesticides); the flame retardants (e.g.; polybrominated diphenyl ethers - PBDEs); and the compounds produced indirectly by the uncompleted burning of the organic matter (e.g.; polycyclic aromatic hydrocarbons - PAH). The majority of these compounds are persistent in the environment and biomagnify in the food chain, especially those most hydrophobic [26–28].

3.2 Heavy metals

The heavy metals are chemical elements and their compounds that occur in the environment naturally in small concentrations (except in sites that have, for example, rock riches in a given element). Lead, cadmium, manganese, aluminum, tetraethyl lead, methylmercury and tributyltin are some examples. Some of them are essential elements, having vital function to organisms or non-essential when have no known function. Both can be toxic or lethal to organisms, depending on their concentrations and sensitivity of the species.

4. Contaminants Studies on Plankton

The ecotoxicological studies using plankton as a tool are usually done in laboratory (assays), in mesocosms or field studies (Table 1). In laboratorial assays, the researcher can control the initial abundance, the target species, the target contaminant and its concentration. Bioassays are simple, sensible, rapid and cost-effective. However, assays often do not represent the real environmental conditions. In field studies, planktonic organisms are collected from the water column and the selection is made mainly by the size of the plankton mesh net. In environmental studies, many contaminants can be present in samples, and is very difficult (sometimes impossible) evaluate the effect of them. The environmental conditions (e.g.; temperature and pH) can also vary and affect planktonic organisms and their relation with pollutants (e.g.; increase absorption). In order to control those conditions without studying in laboratorial assays, researches are done in mesocosms installed in the laboratories or directly into the aquatic environment [29]. However, these type of work should be treated with caution because many studies in mesocosms are not designed with adequate replication and controls [30] especially regarding to difficulties of maintenance and limited space for mesocosms studies. The ecotoxicological studies in mesocosms using plankton as a target taxa are more scarce (e.g.; [31, 32]) than assays or field studies.

4.1 Bioassays

Studies were performed assessing the effects of the pollutants on some planktonic species mainly in freshwater ones [22, 33, 34]. These studies can show the effects of one or few pollutants along time because the effects are associated to a punctual exposure by either one specific or few pollutants. The results obtained in assays can be extrapolated to the environment with caution because, in the environment, the planktonic populations are generally exposed to a mixture of several contaminants [35] in variable conditions (e.g.; temperature, tides, potential predators). The effects of the synergism and antagonism would cause a different response in comparison with the individual pollutants in a controlled environment [35]. Some laboratorial assays done with pollutant contamination in plankton are shown in table 2.

Table 1. Advantages and disadvantages between types of ecotoxicological studies using plankton as target species.

Types	Advantages	Disadvantages
Laboratorial assays	Use of targeted species Use of targeted contaminant Control species abundance Control contaminant concentration	It cannot represent field conditions (natural environment)
Mesocosms	Use of target species Use of target contaminant Control species abundance Control of some environmental conditions	Expensive It needs a suitable space to be installed
Field studies	Represent the real physical-chemical environmental conditions	Expensive Difficult to modelling cause-effect with many variable conditions

Table 2. Main effects of pollutants observed in laboratorial assays on some planktonic species.

Pollutant	Species or group studied	Main effects observed	Reference
PCBs	Marine plankton	Primary productivity reduced; inhibition on the decomposition; drastic and chronic reductions in zooplankton size	[22]
DDT; Fenitrothion; Chlorpyrifos	*Anabaea* sp. (Algae)	Inhibition of the growth (DDT); inhibition of the photosynthesis (fenotrothion); inhibition of CO_2-uptake (chlorpyrifos)	[34]
DDT; Fenitrothion; Chlorpyrifos	*Aulosira fertilissiima* (Algae)	Stimulatory of the growth (DDT); inhibition of the photosynthesis (fenotrothion); inhibition of CO_2-uptake (chlorpyrifos)	[34]
Tributhyltin	*Daphnia magna* (Cladocera)	Obesogen	[33]
pp'-DDE	*Daphnia magna* (Cladocera)	Negative effect on fecundity (chronic exposure)	[24]
Methamidophos (pesticide)	*Brachionus calyciflorus* (Rotifer)	Transgenerational cost of inducible defenses	[36]
Copper	*Thalassiosira aestivalis* (Diatom)	Inhibition of the growth	[37]
Cadmium	*Chaetoceros tenuissimus* (Diatom)	Decrease of growth; genomic changes; apoptosis	[38]

4.2 Fieldworks

Researches on ecotoxicology using plankton have been done in the field. The cause-effect relationship sometimes is difficult to understand because many abiotic and biotic factors can influence on planktonic community, and it may be exposed to several pollutants, that can cause many different effects.

The transference of pollutants into the environment can occur through the organisms *per se* and through their fecal pellets or carapaces from ecdise. Elder and Fowler [39] observed the transference of PCBs from euphausidae (Euphasiacea, Crustacea) to the seafloor through its fecal pellets. Zooplankton may also play a secondary role in decreasing pollutants concentrations to the other trophic levels through phytoplankton bloom, which biodilutes the pollutants in phytoplankton and, consequently, the zooplankton contamination [11]. This was observed for PCBs in a study conducted by Nizzetto et al. [11], but it is reasonable to many pollutants.

The eutrophication process is an important issue in contaminants distribution because promotes a decrease of pollutants concentration in phytoplankton. The increase of nitrogen and phosphorus content lead to increasing the eutrophication through unicellular and colonial planktonic organisms. In a revision done by Mailman et al. [40], the increase of phosphorous was cited as a strategy to lower methylmercury concentration in a reservoir. Eutrophication process could deplete dissolved oxygen and cause a local extinction of sensible species, for example. Therefore, eutrophication can change the environment and the community richness, affecting the pollutants bioaccumulation and biomagnification [6, 11].

The chemical stress caused by pesticide contamination might decrease the efficiency of resource use and the shortening of the food chains because of reduced energy flow to higher trophic levels [41]. Therefore, we observe that the use of plankton in ecotoxicological studies done in the field is very useful, but variables such as phosphorous levels, size of food chain, can be considered to understand the results. Table 3 shows some studies evaluating the contaminants on planktonic communities in the environment.

Table 3. Some contaminants assessed in planktonic communities in field studies.

Pollutant	Plankton size	Studied area	Reference
Microplastics	505 µm	South China sea	[42]
Microplastics	180, 280, and 335 µm	Portugal coast	[43]
Methylmercury	70 and 350 µm	Uatumã river basin (Amazonas, Brazil)	[44]
PCDD/Fs, PCBs and PBDEs	200 µm	Baltic sea	[28]
PCBs	60-200, 200–500, and 500–1000 µm	Marseille bay (Mediterranean sea)	[18]
DDTs and PCBs	200 µm	Como bay (Lake Como, Italy)	[19]
Novel brominated flame retardants, HBCD, and PBDEs	450 µm	Lake Maggiore (Northern Italy)	[45]
DDTs and PCBs	450 µm	Lake Maggiore (Italy)	[20]
Microcystine	20 µm	Jacarepaguá Lagoon (Rio de Janeiro, Brazil)	[46]

5. Bioaccumulation, Biodilution and Biomagnification

Bioaccumulation is the process of pollutants accumulation in the biota. Since planktonic organisms are in the basis of the food web, their bioaccumulation shows the initial steps of the pollutants input in the food web. Phytoplankton accumulates the pollutants mainly directly from water by the adsorption and absorption according to the partition coefficient of each pollutant [11, 15, 47]. Zooplankton incorporates them by ingestion of contaminated algae, suspended particulate matter, other zooplankton organisms, and bacteria or directly by absorption from water [11, 19, 20]. The pollutants also can increase along organism life time, reaching highest concentrations in older organisms as a result of bioaccumulation process along all life. This is very discussed for fish but is not very commonly discussed for planktonic organisms. For example, Kainz, Telmer and Mazumder [48] observed increase of methylmercury concentrations in four size categories of planktonic organisms from some coastal lakes in Vancouver, Canada.

Biodilution is the reduction of pollutants in plankton per unit. The concentration of pollutant decreases in the phytoplankton and, consequently, by transferring it to zooplankton and other trophic levels, their concentrations commonly are also lower [11]. This can occur naturally or induced by human due to algal blooms. Berrojalbiz et al. [49] studied POPs on Mediterranean sea and observed that this biomass dilution was more pronounced for the less hydrophobic compounds. The biodilution is a very important mechanism involved in reduction on pollutants concentration in the environment [49]. Brito et al. [47] showed that the different ways to incorporating methylmercury by phytoplankton and zooplankton caused the occurrence or not of the biodilution in an Amazon lake. Since phytoplankton accumulates mercury directly from water, and zooplankton accumulates it from the water and also by feeding, the increase of phytoplankton biomass decreased its methylmercury concentrations, but did not the zooplankton concentrations [47].

The biodilution can be a strategy to decreasing the pollutant content in the biota in new reservoirs [40]. Mailmann *et al*. [40] shown that the phosphorus addition can be used as a way for decreasing the contaminant since it increases algae and reduces methylmercury per unit of algae. On the other hand, this strategy can induces to eutrophication, and hydroelectric reservoirs usually are already rich in nutrients and depleted in dissolved oxygen. However, this strategy should be carefully debated.

The biomagnification process of contaminants is the increase of contaminants concentration through the food web, achieving the highest concentration in top chain organisms. This can be observed for many pollutants, as the methylmercury and the insecticide DDT, for example, along the food web, including inside the plankton community. Kasper et al. [50], observed in Samuel reservoir (Brazilian Amazon) lower percentages of methylmercury in phytoplanktonic organisms when compared to zooplanktonic ones.

6. Temporal Trends

The planktonic organisms allow an intermediary temporal assessment in aquatic systems, between water and organisms belonging to high trophic levels, such as fish. The matrix chosen is related to witch interval of time is desired to be evaluated. Studies done with water assessments allow observed changes in a short time interval as minutes, hours or days. The plankton studies, in general, do not allow this type of analysis with very short temporal variation. Normally, temporal studies using plankton analyze variation between months or seasons. The variation of concentrations in plankton is dynamic and relatively quick because of their rapid grow and the short lifetime, for several planktonic organisms. Thus, they provide a dynamic response to environmental contamination, being an excellent indicator of seasonal pollution. Therefore, if the assessment of years (years or decades) is the focus of the study, it is necessary to use organisms with a highest life cycle or sediments.

Many studies have used the plankton to temporal assessments of natural processes that occurs seasonally in the environment. In an Amazonian river (Uatumã), the methylmercury concentrations in zooplankton along a year were correlated to naturally changes in the water level of the river [44]. Plankton also can be used to assess changes in the environment that affects the concentration of pollutants in biota, as observed in damming rivers to construct reservoirs [51, 52]. Plankton concentrations are faster modified, and the anthropogenic impact of mercury (e.g.) in these organisms can be observed quickly [53].

7. Barriers in Working

7.1 Sampling

The laboratorial analysis of pollutants is done with a minimum of mass of the sample that, in general, varies according to concentration of pollutant in the sample. Samples more contaminated generally can be analyzed with smaller mass than those less contaminated. The analysis of heavy metals require less mass of sample [44, 54] than the analysis of organic pollutants as pesticides or polybrominated diphenyl ethers [11, 19, 20]. Since density of plankton varies spatially and temporally, obtaining the required mass can be hard and, sometimes, almost impossible. Many hauls of plankton net can be necessary, and a large volume of filtrate sample can be useful.

Another common problem during field sampling is the plankton sample contamination. This can occur mainly due two reasons: with petrol, atmospheric pollution or inadequate bottle decontamination since plankton samples have, in general, low concentrations; and with other things (besides plankton) that can be retained in the plankton net during sampling. Many materials can be retained by the nets such as vegetable debris, microplastics and inorganic particles (e.g.; sandy or clay). Those materials need to be removed from filtrate material to obtain a purer plankton sample (otherwise, the concentration analyzed will not represent the plankton sample but a mixture of plankton and other materials retained). That can be done by filtering the filtrate material in a set of meshes and then rinsing with ultra-pure water. These procedures can remove fine matter (smaller than the meshes size), but for larger particles, a removal with stainless steel tweezers will be necessary. These procedures can be very laborious but will avoid errors with the estimates of pollutant. Another method to separate the filtrate material is through microscopic analysis and its identification. This assessment can express unit or biomass of plankton in the sample. Therefore, these results can be used for the explanation of patterns of pollutants accumulation in specific organisms.

7.2 Analyses

Nowadays, analytical methods increasingly sensible and accurate allow detecting contaminants in low concentrations, like the commonly observed in plankton. The concentration of pollutants in planktonic organisms is quite low because they have a short life cycle, therefore, accumulate pollutants for a short time and because they belong to a low trophic level (to contaminants that biomagnify). Therefore, all procedures need to be conducted carefully in order to avoid losses of sample mass and to avoid contamination of the sample.

7.3 Separation

The common separation of plankton samples in ecotoxicological studies is by mesh of plankton net (and, consequently, size of the organisms). Therefore, in this way, the sample can be a mixture of different species since small animals can be retained in small meshes (< 60 µm) that collect abundantly phytoplankton [55]. In the larger meshes (e.g.; 60, 80 and 120 µm), the separation between phytoplankton and zooplankton generally is less worrisome because without filamentous algae in the site, the collected organisms will be mainly zooplankton (or ichthyoplankton). There are several ways to separate the material to obtain a sample of one kingdom (and not separated only by size). Therefore, they are not efficient in some environments (e.g.; places having too much suspended material or filamentous algae), or for some mesh sizes.

A method described by Behrendt and Krocker [55] is based on two well-known effects; first, the positive phototaxis of zooplankton individuals; and, secondly, the phenomenon that these individuals migrate to the bottom of closed vessels. On the basis of these phenomena, a zooplankton trap is constructed and different migration behaviors of several zooplankton groups occur mainly according to their size [55]. Another way for material separation consist in a light trap used for isolates zooplankton and suspended particles from an environment with a high content of filamentous algae [56]. The planktonic organisms can be separated by addiction of commercial gaseous water to a funnel with the sample that will cause the zooplankton sedimentation (narcotized by water dioxide carbon) and phytoplankton fluctuation [57]. Regardless the method chosen, care should be taken in order to not handle the sample with materials, reagents or in a site that can occur sample contamination.

7.4 Bioassays

When bioassays are done, the effect of different pollutants misture in planktonic organisms normally is not assessed. Therefore, synergisms and antagonisms are not considered in general. The bioassays are generally done with one target species, which is often a model species to toxicological studies (for example, for studies with zooplankton, the most used species are *Daphnia* spp.). That process has a limitation when extrapolation is necessary because each species has a different toxicological response [33, 35].

8. Conclusions

The planktonic community has a fundamental role on pollutants cycle in the environment. Plankton is an excellent tool in the environmental modeling processes about the pollutants cycle, as well as in the modeling of possible effects on the aquatic biota. Since the planktonic organisms have a short life cycle, they are very useful to understand the quick dynamics of the environment. The complexity of their interactions makes the planktonic community a great tool for ecotoxicological assessments. Contaminants can cause several effects in planktonic organisms, such as inhibition of growth, negative effects on reproduction, death of more sensitive species. Those can lead to a drastic change in the composition, abundance and diversity of communities. Consequently, ecosystem processes such as matter production and decomposition can be altered. Therefore, contaminants can affect since individuals until ecosystems where they live. Many ecotoxicological researches have been conducted with this group since better analytical techniques are available.

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