ECOTOX BRASIL
SOCIEDADE BRASILEIRA DE ECOTOXICOLOGIA

Ecotoxicol. Environ. Contam., v. 15, n. 1, 2020, 43-49
doi: 10.5132/eec.2020.01.06

EEC

Effect of lead in the aerobic decomposition of *Myriophyllum aquaticum* (Vellozo) Verdecourt

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Received April 08, 2019; Accept August 31, 2020

Abstract

Lead is a toxic element that has been used since early times and is still employed today in several industrial processes. Events as the collapse of the Fundão dam in Bento Rodrigues, district of Mariana (MG) on November 2015, significantly increase concentrations of metals above-recommended levels, including lead. In this context, this study aims to evaluate the impact of lead in the cycling rate of organic matter in the aquatic environment. Thus, the rates of aerobic decomposition of *Myriophyllum aquaticum* (Vell.) Verdec at different concentrations of lead (5.0, 10.0, 20.0 and 30.0 mg L^{-1}) were measured, analyzing the dissolved oxygen consumption. Decomposition chambers filled with samples of water and *M. aquaticum* were incubated at 20° C in the dark for 80 days and periodically had the oxygen concentrations determined by polarography, when the concentrations were less than 2 mg L^{-1}, the incubations were re-aerated. At the lowest concentrations (5.0 and 10.0 mg L^{-1}) of lead the mineralization was lower, however, the reaction coefficients and the amount of oxygen consumed were equivalent to the control. At the highest concentrations (20.0 and 30.0 mg L^{-1}) the mineralization was increased, with the reduction of reaction coefficients and higher oxygen consumption.

Keywords: aquatic macrophytes; contamination, oxygen uptake; metal; mathematical modeling.

INTRODUCTION

Heavy metals and others elements may correspond to trace elements in biochemical reactions, but when present in large quantities in the cells they form nonspecific complex compounds (Nies, 1999), they have the capability to undergo bioaccumulation and biomagnification processes (Salgado *et al*., 2018) and all enzyme systems are potentially susceptible to metal toxicity (Moreira & Moreira, 2004). Most of these metals are transitional elements and do not have a complete d orbital, allowing the formation of cations able of creating complex compounds that may or may not participate in oxide-reduction reactions (Nies, 1999). Cations of heavy metals, especially those with high atomic numbers, tend to bind to -SH groups (sulfhydryl group) and may inhibit the activity of bound enzymes, for example, the oxidative phosphorylation or interact with ions such as Cd^{2+}, Zn^{2+}, Ca^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+} or Mg^{2+}, thus inhibiting their physiological functions (Nies, 1999). Metals are predominantly soluble in the ionic form or chelates of organometallic compounds and their solubility in water allows the absorption by the organisms (Sigg, 1985; Landis & Yu, 2003).

Lead has been used for a long time; there are indications of its use 7000 years ago by the Egyptians (Hoffman, 1995). Some historians believe that the intoxication of the aristocracy by lead may have been responsible for the destruction of the Roman culture (Hoffman, 1995) since it was used as a wine sweetener (lead acetate) in water pipes and kitchen containers (Baird, 2007). The entrance of lead in aquatic environments is due to atmospheric deposition, sewage discharges and direct industrial and mine discharges, soil leachate, mine tailing, highway runoff, and lead plumbing systems (Hoffman, 1995). In these environments the lead can be found in the sediments as in the aqueous portion, this depends on the pH and salts

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dissolved and organic complexing agents present (Azevedo & Chasin, 2003). In surface water lead can be found complexed with organic compounds, such as humic or fulvic acids (Azevedo & Chasin, 2003).

Events such as the disruption of the Fundão tailing containment dam in the Bento Rodrigues district in Mariana (Minas Gerais State, Brazil) on November 5, 2015, where about 50-60 million m³ of sludge was dumped, significantly increase the concentrations of metals above recommended levels in Doce River, including lead (Carvalho *et al*., 2017). The dam rupture ended up transporting large quantities of trace elements and sediment over long distances, in the case of Mariana, 74 million tons were transported in the fourteen months following the disruption while they were to be 2.5 million tons (Magris *et al*., 2019). Despite of not being considered toxic, the mud affected the quality of the soils (Guerra *et al*., 2017), the benthic community (Gomes, *et al*. 2017), macrophyte growth (Bottino *et al*., 2017) and water quality along the river (Hatje *et al*., 2017), besides losses in fishing, energy generation, and water supply (Burton & Nedric, 2017). The concentration of Pb found in the soil was 20.2 ± 4.6 mg kg^{-1} being mainly associated with oxyhydroxides of iron (Queiroz *et al*., 2018); in the Doce River basin, there were rivers stretches where the maximum concentration of Pb were 1.0 mg L^{-1} (IGAM, 2017). In these aquatic environments, macrophytes can play an important role in maintaining ecosystem services, such as nutrient cycling, pollutant retention and biodiversity conservation (Lacoul & Freedman, 2006). These plants affect the physical and chemical characteristics of water and sediment, being a source of organic matter, modifying the spatial structure of the ecosystem and participating in the cycling of nutrients, changing the dynamics of nutrients and communities such as creating habitats for fish, affecting the phytoplankton community (Thomaz & Cunha, 2010; Thomaz & Esteves, 2011). Coarse particulate organic matter derived from macrophytes decomposition can be important source of energy and nutrients in continental aquatic ecosystems (Moore *et al*., 2004).

Owing the interactions of lead in living beings as mentioned, and consequently in the functioning of ecosystems (aquatic and terrestrial), and the importance of macrophytes to aquatic environments, this study aims to evaluate the effect of Pb on the aerobic decomposition of aquatic plants detritus, a fundamental process for the energy flow and for the maintenance of the metabolism of aquatic ecosystems under similar conditions to those of environmental accidents (Doce River). For this purpose, the macrophyte species *Myriophyllum aquaticum* (Vell.) Verdec was selected as organic matter resource, observing whether there would be a negative effect on the metabolic activity of the decomposing microorganisms (e.g., oxygen consumption). Due to the interference of Pb in biochemical routes of microorganisms, it is assumed that lead toxicity will prejudice aerobic decomposition, which should be explained by the decrease in oxygen consumption coefficients and altered accumulated oxygen consumption.

MATERIALS AND METHODS

The water and macrophyte samplings were carried out at the Monjolinho Reservoir located at the Federal University of São Carlos, São Carlos, SP, Brazil (21° 59' S and 47° 52' W). It is a small (ca. 4.69 ha) and shallow (average depth of 1.5 m) artificial environment located in the subtropical region (Santos *et al*., 2009). With two annual climatic regimes, the dry season between April and September, and the warm rainy season between October and March according to Köppen`s systematic (Cwb) (Köppen, 1931). The retention time depends on these seasons and varies from 2.1 to 22.9 days (Santos *et al*., 2009). According to Santos *et al.* (2011), the lacustrine region of the reservoir is characterized by an annual average of suspended material of 48.8 ± 10.3 mg L^{-1}, 11.9 ± 10.7 NTU of turbidity, mean average temperature of 20.6 ± 3.3 °C, and dissolved oxygen of 6.9 ± 0.8 mg L^{-1}. The pH was neutral (6.7 ± 0.3) and the electrical conductivity was 41.6 ± 5.1. The concentration of dissolved inorganic carbon was 4.6 ± 1.3 mg L^{-1} and organic was 2.4 ± 1.1 mg L^{-1}.

The macrophyte used in this study due to its presence in the Monjolinho reservoir was *Myriophyllum aquaticum* (Vellozo) Verdecourt (Haloragaceae), a genus with many species (ca. 68 species) of the aquatic core-eudicots (Silva *et al*., 2011). Known as parrotfeather, this plant occurred in stagnant or slow environment that may or may not be rooted (Silva *et al*., 2011). Native to the plains of central South America but has been widely distributed due to its use in aquarism and is considered invasive in several regions, which is facilitated by the high capacity of regeneration of its fragments (Francis, 2012).

The proposed decomposition experiments were adapted from the biochemical oxygen demand tests (BOD). Thus, through oxygen consumption by cellular respiration (e.g., $C_6H_{12}O_6 + 6\ O_2 \Rightarrow 6\ CO_2 + 6\ H_2O$), thus, it was possible to estimate indirectly the quantity of labile organic matter consumed. This value was calculated by the difference between the initial and final dissolved oxygen concentrations of the sample (Valente *et al*., 1997). To prepare the experiment, the water (≈ 10 liters) was filtered with a cellulose acetate membrane (pore Ø = 0.45 µm, Millipore). The collected macrophytes were washed to remove the periphyton and sediment particles adhered; then, the plants were dehydrated (40 ºC), until constant mass. The C contents of the plant tissues, at the beginning of the experiment, were determined by TOC analyzer (Shimadzu, model SSM-5000A).

The sample water was separated into 24 BOD bottles (ca. 300.0 ml), 6 of them with only water to measure the oxygen consumption due to the decomposition of the dissolved organic matter and eventual phytoplankton cells. In another 6 flasks, samples of water and macrophyte (100.0 mg, dry mass basis, DM) were placed. In the remaining bottles, 100.0 mg (DM) of the macrophyte was added and at different concentrations of lead (5.0, 10.0, 20.0, 30.0 mg L^{-1}) chosen to simulate the scenario of Pb contamination accidents. After, the flasks were aerated (ca. 3 hours), and then, incubated at 20ºC; near the annual temperature average of the reservoir (Santos *et al.*; 2011), in the dark, in order to avoid the phytoplanktonic photosynthesis. Periodically, the concentrations of dissolved oxygen were determined by polarography (oximeter, YSI model 58), and when concentrations were close to 2.0 mg L^{-1}, the incubations were re-aerated again.

After the incubation time (80 days) the contents of the chambers were filtered through cellulose acetate membrane (pore Ø = 0.45 μm; Millipore). The particulate fractions were incinerated at 550° C (two hours) in order determinate the particulate organic matter (POM) and then, those values were multiplied by factor of 0.465 (Santos *et al.*, 2009) to estimate the carbon content (POC). The final concentrations of dissolved carbon were determined by the TOC analyzer (Shimadzu model TOC-L).

Initially, it was assumed that the time consumption of oxygen was defined by first-order kinetics (Equations 1 to 8), as in the BOD tests (Borsuk & Stow, 2000; Davis & Cornwell, 2008).

$$\frac{dL_t}{dt} = -kL_t \tag{1}$$

where Lt = oxygen uptake at time t (mg L^{-1}); k = deoxygenation rate constant (d^{-1}).

Rearranging Equation 1 and integrating results:

$$\frac{dL_t}{L_t} = -kdt \tag{2}$$

$$\int_{L_0}^{L} \frac{dL_t}{Lt} = -k \int_0^t dt \tag{3}$$

$$\ln \frac{L_t}{L_0} = -kt \tag{4}$$

$$L_t = L_0 \times e^{-kt} \tag{5}$$

$$OC = L_0 - L_t \tag{6}$$

$$OC = L_0 - L_0 \times e^{-kt} \tag{7}$$

$$OC = OCmax \times (1 - e^{-kt}) \tag{8}$$

where: OC = oxygen consumption (mg); $OCmax$ = maximum oxygen consumption (mg); e = base of natural logarithm; t = time (day).

The daily rates of dissolved oxygen consumption were integrated and the kinetic parameters ($OCmax$ and k) were determined. For this, the curve fits were made through non-linear regressions, using Levemberg-Marquardt's iterative algorithm (Press *et al.*, 2007). The accumulated oxygen consumption was subjected to the normality test and, in sequence, data were compared by the Kruskal-Wallis test, followed by Dunn's Multiple Comparison Test, since the values of oxygen consumption exhibit a non-normal distribution. A level of $P < 0.05$ was adopted in all comparisons.

The percentage of mineralized carbon (MC) in each treatment was calculated as follows:

$$MC = POCi - (POCf + DOCf) \tag{9}$$

where: MC = mineralized carbon (%); POCi = initial particulate organic carbon (mg); POCf = final particulate organic carbon (mg); DOCf = final dissolved organic carbon (mg).

The calculation of mineralized dissolved organic carbon (MCOD) was performed with the following equation:

$$MDOC = \frac{[(POCi * 0.037) - DOCf] * 100}{POCi * 0{,}037} \tag{10}$$

where: MCOD = mineralized dissolved organic carbon (%); POCi = initial particulate organic carbon (mg); DOCf = final dissolved organic carbon (mg); 0.037 = approximate fraction of dissolved organic carbon (Grando, 2018).

For the calculation of the mineralization coefficient (km) was used:

$$k_m = \frac{Ln\,(^{MC}/_{100})}{t} \tag{11}$$

where: km = mineralization coefficient (day^{-1}); MC = mineralized carbon (%); t = time (day).

RESULTS

The accumulated oxygen consumption only of the water samples of the reservoir were ca. 11.0 mg L^{-1}, thus, for all treatments (Figure 1), this value was subtracted before the transformation of oxygen consumption per g of plant dry weight. The results of accumulated oxygen consumption of the experiments, in general, showed higher consumption in the beginning of the experiment (ca. 10 days). Then, they decreased and maintained between 0.0 and 5.0 mg L^{-1} per day in the control and between 0.0 and 10.0 mg L^{-1} per day in the treatments with Pb.

The statistical tests showed that the oxygen consumption in the control was equivalent to the treatments with 5.0, 10.0, and 30.0 mg L^{-1} of Pb, whereas the incubation with 20.0 mg L^{-1} of Pb was different from all the others treatments ($p < 0.001$). By fitting the oxygen consumption curves, it was possible to observe that in high concentrations of lead there was an increase in the oxygen consumption demonstrated by the increase of the OCmax and a tendency to decrease the values of the oxygen consumption coefficient.

In the lower concentrations of Pb (5.0 and 10.0 mg L^{-1}) there was a decrease in the yield of mineralized carbon (MC) evidenced by the decrease global coefficient of mineralization of organic carbon (km); Table 1. At the highest concentrations of Pb (20.0 and 30.0 mg L^{-1}) there was an increase in the percentage of mineralized carbon and this process was faster than the control itself. There was a gradual increase in DOC mineralization with increasing Pb concentration, at 20.0 mg L^{-1} concentration it was 6.23 times faster than the control and at 30.0 mg L^{-1} it was 4.76.

According to the (linear) relationship between OCmax and mineralized carbon (Figure 2), the angular coefficient pointed out that 3.052 molecules of oxygen were used for each atom of mineralized carbon.

DISCUSSION

The toxicity of lead is explained by its action in fundamental biochemical processes, since it is able to: i) inhibit or mimic calcium, competing in several biochemical processes such as, the activation of calmodulin involving binding with carboxyl groups and in mitochondrial respiration; ii) interfering with the functioning of cell membranes; iii) restrains or stimulate proteins due to the formation of stable complexes with ligands capable of donating electrons such as oxygen (-OH), nitrogen ($-NH_2$), phosphorus ($-H_2PO_3$) and sulfur (-SH), which in this case is bound with the activation of the protein kinase C (Moreira & Moreira, 2004) and iv) reduces the ability of t-RNA to bind to ribosomes (Landis & Yu, 2003). Overall, these biochemical processes interfere with the microbial metabolism (Roane *et al.*, 2009) and consequently, in the cycling of elements in the aquatic environments.

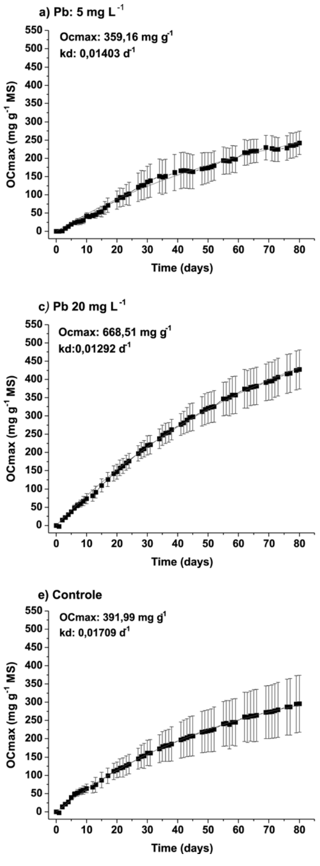

Figure 1. Kinetics of accumulated oxygen consumption owing to the aerobic mineralization of *Myriophyllum aquaticum*.

The detritus of the macrophytes is predominantly composed of refractory organic matter and due to this, the mass decrease observed in these experiments is basically related to the aerobic mineralization of the labile fractions (particulate or dissolved) and consequently to oxygen consumption (Bianchini Jr. *et al.*, 2011). According to decomposition experiments, it was verified that three possible routes of mineralization occur in the aerobic decomposition: i) the rapid oxidation of the labile compounds, that is, it has a short half-life; ii) leaching and catabolism of dissolved organic matter (DOM) which normally have an intermediate half-life; iii) oxidation of refractory particulate debris (POM) that have a long half-life (Bianchini Jr., 2003). These routes are influenced by several factors such as the types of metabolism that perform the decomposition, environmental variables such as temperature and dissolved oxygen, among others, the characteristics of the detritus, for example, chemical composition, particle size and forms by which the debris is adduced or generated (Bianchini Jr., 2003).

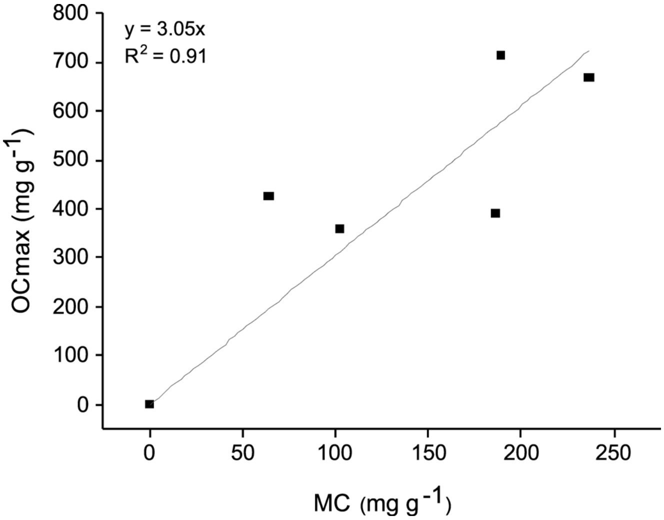

Figure 2. Relationship between maximum oxygen consumption (OCmax) and mineralized carbon (MC) from aerobic decomposition of *Myriophyllum aquaticum*.

Table 1. Parameters of *Myriophyllum aquaticum* aerobic mineralization; where: i) initial and final particulate organic carbon (POCi and POCf, respectively); ii) final dissolved organic carbon (DOCf); iii) carbon mineralized (MC); iv) mineralization coefficient (km); v) half-life of *M. aquaticum* mineralization (t½); vi) dissolved organic carbon mineralized (MDOC), DOC mineralization rate constant (km DOC); vii) half-life of DOC mineralization (t½).

Pb ($mg L^{-1}$)	**POCi** (mg)	**POCf** (mg)	**DOCf** (mg)	**MC (%)**	**km** (d^{-1})	**t½** (d)	**MDOC** (%)	k_M**DOC** (d^{-1})	**t½** (d)
0.0	40.65	21.59	1.078	44.34	0.0073	94.6	28.32	0.0042	166.5
5.0	39.22	28.77	0.911	24.32	0.0035	199.0	37.23	0.0058	119.1
10.0	38.62	31.89	0.849	15.24	0.0021	335.4	40.61	0.0065	106.4
20.0	40.86	17.68	0.189	56.31	0.0104	67.0	87.50	0.0260	26.7
30.0	40.57	22.08	0.308	45.05	0.0075	92.6	79.50	0.0198	35.0

The parameters of oxygen consumption, regardless of the type of macrophyte, are derived from the qualitative characteristics of the plant tissues (such as *Myriophyllum aquaticum* biomass), as well as the proportion of each fraction of organic matter (Bianchini Jr., 2003). The high oxygen consumption in the first days (Figure 1) can be explained by the consumption of the labile-soluble portion (23.76%) of the organic matter and gradually decreased due to the predominance of the mineralization of the refractory fraction (76.24%) of the macrophyte (Bianchini Jr. *et al*., 2011).

Experiment with macrophytes from an oligo-mesotrophic reservoir showed that the amount of oxygen used in the degradation ranged from 205.0 mg g^{-1} (*Eleocharis* sp) to 533.0 mg g^{-1} (*Ludwigia* sp); overall, the values of OCmax obtained in our study were within this recorded range. However, in the experiment with macrophytes of the Piraju Reservoir (Bianchini Jr. *et al*., 2011), the mineralization of *Myriophyllum aquaticum* presented much lower value (290.0 mg g^{-1}) than was observed in our results. The value of OCmax from the control incubation was probably higher due to the trophic state of the Monjolinho reservoir (eutrophic; Santos *et al*., 2011) that favored the degradation of the detritus (Viaroli & Christian, 2004).

Observing the data in Figure 1, the decrease of kd and increase of the OCmax identified in the two treatments with the highest concentration of Pb (20.0 and 30.0 mg L^{-1}); indicated that the presence of lead led to decreased reaction rates (i.e., lower speed) and increase in the amount of oxygen consumed. In that case, without the presence of lead, it was estimated that they were oxidized ca. 35% of the plant carbon. If the stoichiometry ratio (3.052; Figure 2) was maintained, the addition of lead increased the carbon oxidation to 30.01% (Pb: 5.0 mg L^{-1}), 36.07% (Pb: 10.0 mg L^{-1}), 53.60% (20.0 mg L^{-1}) and 57.72% (30.0 mg L^{-1}). It is important to note that the value obtained experimentally was higher than the theoretical value, usually adopted (2.66; Bianchini Jr. *et al*., 2011) for the calculation of the oxidation of organic matter based on glucose. These changes suggest a probable alteration in the metabolic routes related to the aerobic oxidation of the organic matter. In organisms, the lead can disturb the pathways of oxidative phosphorylation, metabolic reactions by which aerobic organisms synthesize ATP through the transport of electrons by complexes in the mitochondrial membrane (Vallee & Ulmer, 1972). The catabolism of organic matter may follow three different pathways, the citric acid cycle or Krebs cycle, the glyoxylate cycle, and the pentoses pathway. In the Krebs cycle, the aerobic reactions are able to release ATP through the oxidation of glucose into carbon dioxide and water. The glyoxylate cycle is observed in some microorganisms, in this way Acetyl-CoA condenses with the oxaloacetate forming citrate. The pentoses pathway (or phosphoglycate pathway), is the route where glucose-6-phosphate is oxidized to pentoses-phosphate (Nelson & Cox., 2014).

The amount of oxygen consumed may change due to the metabolic pathway, since there are enzymes in which lead has an inhibitory interaction, such as the pyruvate dehydrogenase complex that transforms pyruvate into Acetyl-CoA and the succinate dehydrogenase responsible for the dehydrogenation of succinate to fumarate, both in the citric acid cycle, glucose-6-phosphate dehydrogenase that dehydrogenates glucose-6-phosphate into 6-phosphoglycate in the pentoses phosphate pathway (Vallee & Ulmer, 1972; Nelson & Cox, 2014).

Differences in the OCmax and kd can also be explained due to degradation in a greater or lesser amount of other substances present in the tissues of the macrophyte used (Table 2) as well as monosaccharides (e.g., polyphenols, terpenes, fatty acids), since the composition (Bianchini Jr. *et al*., 2011).

The method of depletion of the dissolved oxygen (DO) concentrations evaluates the mineralization, indirectly, through the consumption of dissolved oxygen using the following Equation 12 (Bianchini Jr. *et al*., 2003). Each component has an O/C ratio as shown in Table 3.

$$C_xH_{2y}O_z + \left[x + \left(\frac{y-z}{2}\right)\right] O_2 \Rightarrow xCO_2 + yH_2O \qquad (12)$$

Besides the decomposition of these substances consume different amounts of oxygen, the catabolism of these generates secondary compounds that interfere in the stoichiometric coefficients since they are also used in the reactions of biosynthesis and energy acquisition (Bianchini Jr. *et al*., 2011).

Lead also interacts with organic matter (particulate and dissolved) by adsorption, ion exchange reactions or complexation that, in this case, interferes in the geochemistry of the metal ions, changing its dissolution, charge and redox potential. Its connection with humic substances depends not only on the concentration of the metal or organic matter but also on the ionic strength of the ions (Bezerra *et al*., 2009). Thus, in lower concentrations of lead, the oxygen consumption was similar, probably due to the interaction between organic matter and Pb, attenuating the toxic action of this metal in the microorganism metabolism. Thus, the effective concentrations of lead for the mineralization were smaller than the nominal ones.

The decomposition depends, in addition to abiotic factors, on the organisms responsible for these processes;

Table 2. Composition of *Myriophyllum aquaticum* (Little, 1979; Martínez-Yáñez *et al*., 2018).

Macrophyte composition	% Dry mass	Macrophyte composition	% Dry mass
Ash	17.25	S	0.40
Crude protein	22.30	Cl	1.45
Crude fiber	14.55	Na	0.80
Dry matter	10.65 ± 2.13	Mg	0.75
Organic matter	88.13 ± 0.13	K	1.90
Hemicellulose	19.26	Mn	0.50
Cellulose	15.39	Fe	0.10
Lignin	5.14 ± 1.05	P	0.50
Extract of ether	4.25 ± 0.35	SiO_2	2.45
Dry matter	7.5	Ca	2.95

Table 3. O/C proportion of some compounds (Marzzoco & Torres, 1999; CAS, 2019).

Compounds	Formula	O/C
Monosaccharides	$(CH_2O)n$	2.7
Glyoxylic acid	$C_2H_2O_3$	1.33
Lignin	$C_9H_{10}O_2, C_{10}H_{12}O_3, C_{11}H_{14}O_4$	ca. 3.03 – 3.11
Cellulose	$(C_6H_{10}O_5)n$	2.66
Palmitic acid	$C_{16}H_{32}O_2$	3.88

they use specific metabolic pathways that produce different intermediates that interact with other organisms and compounds (Bianchini Jr. *et al*., 2011). The growth of these organisms during the decomposition conserves carbon in the organic form, which alters the circulation of organic compounds, changing also the stoichiometries relation (Cunha-Santino & Bianchini Jr., 2002).

Some studies have shown that a significant increase in the concentrations of metals leads to marked responses, thus increasing the resistance of the microorganisms (Roane *et al*., 2009). This fact may explain the increase in OCmax, kd and increased mineralization at the highest concentrations (20.0 and 30.0 mg L^{-1}), whereas the lowest concentrations (5.0 and 10.0 mg L^{-1}) had lower values than the control itself. The response to metal toxicity depends on the type of metal, its concentration on the medium, its availability and the type of microorganism (Baquero & Negri, 1997). The variability of the mechanisms of resistance of these organisms is derived from the diversity of genotypes and the various selective pressures, and with their large population numbers and genomic diversity, the microorganisms have great adaptability (Muñoz *et al*., 2012).

Considering the experimental procedures adopted, we found that in lower concentrations of lead, there was a negative effect on the cycling rates of *M. aquaticum* detritus in the aquatic environment. Due to the various types of possible interference (*e.g*., in catabolic routes, adsorption, selection of microorganisms), the presence of lead promoted changes in reaction rates, as well as in the amount of oxygen consumed. In general, the increase in Pb concentration tended to increase the amount of oxygen consumption, as well as the DOC mineralization rates.

ACKNOWLEDGEMENTS

The authors thank Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP process nº 17/20722-1) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq process nº 305263/2014-5) for the granting of scholarships.

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