**Effects of anthropogenic heavy metal contamination on litter decomposition in streams – a meta-analysis**

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**Abstract**

Many streams worldwide are affected by heavy metal contamination, mostly due to past and present mining activities. Here we present a meta-analysis of 38 studies (reporting 133 cases) published between 1978 and 2014 that reported the effects of heavy metal contamination on the decomposition of terrestrial litter in running waters. Overall, heavy metal contamination significantly inhibited litter decomposition. The effect was stronger for laboratory than for field studies, likely due to better control of confounding variables in the former, antagonistic interactions between metals and other environmental variables in the latter or differences in metal identity and concentration between studies. For laboratory studies, only copper+zinc mixtures significantly inhibited litter decomposition, while no significant effects were found for silver, aluminum, cadmium or zinc considered individually. For field studies, coal and metal mine drainage strongly inhibited litter decomposition, while drainage from motorways had no significant effects. The effect of coal mine drainage did not depend on drainage pH. Coal mine drainage negatively affected leaf litter decomposition independently of leaf litter identity; no significant effect was found for wood decomposition, but sample size was low. Considering metal mine drainage, arsenic mines had a stronger negative effect on leaf litter decomposition than gold or pyrite mines. Metal mine drainage significantly inhibited leaf litter decomposition driven by both microbes and invertebrates, independently of leaf litter identity; no significant effect was found for microbially driven decomposition, but sample size was low. Overall, mine drainage negatively affects leaf litter decomposition, likely through negative effects on invertebrates.

Key-words: contamination origin, decomposer, litter type, metal identity, study type.

**Capsule**

Heavy metals have negative effects on litter decomposition, but magnitude of the effect depends on methodological and environmental conditions.

**Highlights**

A meta-analysis was done to assess the effects of heavy metals on litter decomposition.

Heavy metals significantly and strongly inhibited litter decomposition in streams.

The magnitude of the effect depended on methodological and environmental conditions.

The effects were significantly stronger for laboratory than for field studies.

Mine drainage inhibited leaf (not wood) and total (not microbial) decomposition.

**Introduction**

Watersheds worldwide are generally dominated by small forest streams (Allan and Castillo, 2007). In these shaded streams, the decomposition of organic matter of terrestrial origin is a fundamental ecosystem process (Wallace et al., 1997). The mineralization of this organic matter (henceforth called litter) and its incorporation into aquatic food webs are mediated by the activities of microbial decomposers and invertebrate detritivores (Hieber and Gessner, 2002). Thus, changes in community composition or activity of these organisms may affect the rate at which litter is decomposed, with consequences for energy, carbon and nutrient cycling, which may jeopardize the services these systems provide to human societies (Covich et al., 2004).

Streams worldwide are exposed to a multitude of stressors, which may negatively affect aquatic communities and ecosystem processes (Young et al., 2008). One of these stressors is heavy metal contamination, which is prevalent in areas where active or abandoned mines exist, but can also be caused by motorways as well as by industrial and agricultural activities (Hogsden and Harding, 2012; Woodcock and Huryn 2005). Heavy metal contamination has been shown to negatively affect aquatic communities and litter decomposition in small forest streams (Bermingham et al., 1996; Hogsden and Harding, 2013; Niyogi et al., 2002; Scheiring, 1993), but the magnitude of the effects may vary depending on the communities involved in litter decomposition, litter quality, origin of metal contamination, metal identity, and/or type of study.

The main microbial decomposers in running waters are aquatic hyphomycetes (Gulis and Suberkropp, 2003). Exposure to heavy metals tends to reduce their reproductive activity and growth, but the magnitude of the effect depends on fungal species identity and origin, and metal identity and concentration (Abel and Bärlocher, 1984; Azevedo and Cássio, 2010; Duarte et al., 2004, 2008; Jaeckel et al., 2005; Miersch et al., 1997; Moreirinha et al., 2011). Some aquatic hyphomycetes are very efficient at producing metal-binding proteins, which allow them to tolerate some degree of metal contamination (Braha et al., 2007; Guimarães-Soares et al., 2006, 2007; Jaeckel et al., 2005; Miersch et al., 1997) and explain their presence in heavily polluted streams (Sridhar et al., 2000). Differences in heavy metal tolerance may affect hyphomycete community structure in contaminated environments (Batista et al., 2012; Duarte et al., 2004, 2008, 2009; Moreirinha et al., 2011; Niyogi et al., 2009). Changes in fungal community structure and decreases in activity can lead to reduced rates of litter decomposition if tolerant species are not able to compensate for the loss of sensitive species (Batista et al., 2012; Duarte et al., 2004, 2008, 2009; Moreirinha et al., 2011), but some functional redundancy among species may also exist (Gonçalves et al., 2011).

Heavy metal contamination also affects community structure and activity of invertebrates through multiple pathways (reviewed by Hogsden and Harding, 2012). Some invertebrates, including detritivores, are highly sensitive to metal contamination of stream water and coating of sediments with metal hydroxides. This sensitivity leads to distinct community structure and biomass in metal contaminated and non-contaminated streams, with the former generally having less diverse communities that are dominated by a few tolerant taxa (Abel and Bärlocher, 1988; Carlisle and Clements, 2005; Chaffin et al., 2005; Hogsden and Harding, 2013; Niyogi et al., 2001, 2002). Contamination of litter, either through plant bioaccumulation of heavy metals from the soil or through metal adsorption after submergence, can also affect detritivores by decreasing consumption and growth rates and increasing mortality (Abel and Bärlocher, 1988; Campos et al., 2014; Gonçalves et al., 2011). Distinct fungal species have different degradative capabilities and elemental composition (Canhoto and Graça, 2008; Cornut et al., 2015; Danger and Chauvet, 2013). Thus, changes in microbial community structure and activity induced by heavy metal contamination can inhibit litter consumption by detritivores (Arce Funck et al., 2013; Batista et al., 2012; Gonçalves et al., 2011). Under field conditions, however, these pathways occur simultaneously and their relative importance in determining the effects of heavy metal contamination on stream invertebrates is difficult to quantify. Nevertheless, litter decomposition mediated by the activities of detritivores should be inhibited in heavy metal contaminated streams, and given that the activity of detritivores depends partially on microbial colonization of litter, this inhibition should occur to a larger extent than that observed for microbially mediated litter decomposition (Medeiros et al., 2008). Similarly, inhibition of litter decomposition by metal contamination should be mainly driven by changes in detritivore rather than in microbial activity (Chaffin et al., 2005; Niyogi et al., 2001).

Detritivores usually prefer high quality litter (e.g. with low toughness and carbon:nutrients ratios), and generally colonize submerged litter only after its palatability has been increased by the activities of microbes that macerate the litter and increase its nutrient concentration (Canhoto and Graça, 2008; Graça et al., 2001). Thus, the relative contribution of detritivores and microbes to litter decomposition depends on its quality, with a higher relative contribution of detritivores to the decomposition of high quality than to that of low quality litter (Gulis et al., 2006; Hieber and Gessner, 2002). This, together with the information presented above, suggests that heavy metal contamination may affect the decomposition of high quality litter to a greater extent than that of low quality litter (Bermingham et al., 1996).

Metal contamination in streams may occur in isolation, such as from some industries, or co-occur with other stressors. With mine drainage, there are several stressors that can affect stream biota and processes: toxicity of dissolved metals, acidity, and deposition of metal precipitates (McKnight and Feder, 1984). In many cases with mine drainage, heavy metal pollution is associated with acidic pH (Hogsden and Harding, 2012) which is due to reactions that produce sulfuric acid from pyrite weathering. The resulting degree of acidity of mine drainage is also influenced by the amount of buffering from carbonates (e.g., limestone) in the local geology. Thus, pH of mine drainage can vary from acidic to neutral, depending on the mine and its local geology. Low pH can directly affect stream organisms or their activity (Cornut et al., 2012). For instance, low pH inhibits pectin degrading enzymes, negatively affecting the degradative capabilities of microbes (Suberkropp and Klug, 1980). Acidity can also play an important role in the effect of heavy metal contamination on aquatic communities and litter decomposition. Acidic conditions promote metal solubilisation while higher pH can induce the formation of metal hydroxide precipitates (Hogsden and Harding, 2012), which can differentially affect aquatic microbes and invertebrates (Niyogi et al., 2001). In addition, certain mines, primarily those for production of metals as opposed to coal, usually have higher concentrations of toxic metals such as copper and zinc, and the identity of metals at a site will be related to the local geology.

Metal identity can also be an important factor moderating heavy metal contamination effects on aquatic communities and litter decomposition (Duarte et al., 2008, 2009; Medeiros et al., 2010; Pradhan et al., 2011). In laboratory studies, copper (Cu) has been reported to be more toxic than zinc (Zn) to microbial communities (fungal diversity and community structure) and microbially driven litter decomposition (Duarte et al., 2008, 2009), corroborating studies reporting that Cu is more toxic than Zn to several species of aquatic fungi (Azevedo et al., 2007; Guimarães-Soares et al., 2007). In addition, the effects of nanocopper oxide (CuONP) and ionic Cu appear to be stronger than those of nanosilver (AgNP) and its ionic form (Ag) on litter decomposition, which were also accompanied by highest inhibitions on bacterial biomass, fungal diversity, reproduction and stronger alterations on microbial community structure (Pradhan et al., 2011). In a microcosm study by Medeiros et al. (2010), iron (Fe) affected fungal diversity and community structure more than Zn or manganese (Mn), but no differences were found on litter decomposition among microcosms exposed to the different metals. However, the experiment ran for only 16 days and the exposure time is also reported to influence the effects of heavy metals (e.g. Duarte et al., 2004, 2008), with stronger inhibitions being found on microbially driven litter decomposition after longer periods of exposure (e.g. 25 vs. 13 days, Duarte et al., 2004; 40 vs. 10 or 25 days, Duarte et al., 2008).

Factors potentially moderating the effect of heavy metal contamination on aquatic communities can be better isolated and controlled in laboratory experiments than in field observational studies, with field manipulative studies lying in between (Woodward et al., 2010). Thus, a stronger effect of heavy metal contamination on litter decomposition is expected in laboratory experiments, as shown previously for the effect of nutrient enrichment on litter decomposition (Ferreira et al., 2015).

Analysis of variation in the effect of heavy metal contamination on litter decomposition among studies due to differences in methodology and environmental conditions could reveal the moderators of the response of this key aquatic process to heavy metal contamination. However, despite numerous studies addressing the effects of heavy metal contamination on litter decomposition being conducted since the late 1970s, no systematic review of this literature has been performed to date to integrate results and allow broad conclusions to be drawn. Here, we carried out a meta-analysis based on 38 primary studies to assess the overall effect of heavy metal contamination on litter decomposition and, most importantly, to identify methodological and environmental variables that can explain variation in the magnitude of the effect among studies.

**Material and methods**

*Literature search and selection of relevant primary studies*

We searched for primary studies published between January 1970 and October 2014 that addressed the effect of heavy metal contamination on litter decomposition in streams. The search was done using Google Scholar, personal literature databases and reference lists in primary studies and in review papers. Combinations of the following search terms were used in Google Scholar: (decomposition or processing or breakdown or decay) and (litter or leaf or leaves or bark or wood) and (metal or ‘metal name’ or mine or mining or acid drainage) and (stream or river or water course or laboratory or microcosm).

To be included in the analysis, primary studies had to : (i) explicitly address the effects of chronic (rather than episodic) heavy metal contamination on litter decomposition, (ii) focus on effects of heavy metal contamination due to past or present anthropogenic activities (as opposed to that of natural origin), (iii) focus on running waters (i.e. rivers, streams, artificial flowing channels, laboratory microcosms with agitation) rather than standing waters (e.g. wells), (iv) in the case of laboratory studies, consider litter decomposition driven by microbial assemblages (as opposed to individual species), (v) compare litter decomposition rates for at least one non-contaminated (reference) and one equivalent contaminated condition, (vi) report rates of decomposition of litter of allochthonous origin (i.e. grass or tree leaves or woody substrates) rather than litter derived from macrophytes or artificial substrates such as cotton strips or cellulose substrates, and (vii) report sample size (n) and a measure of variation (SE, SD, 95% CL; not necessarily mandatory) for both reference and contaminated conditions. The final database included 38 studies that satisfied the above inclusion criteria and contributed 133 unique cases to the database (references marked with an ‘\*’ in the References list).

*Effect size*

In most cases, litter decomposition was reported as the exponential decomposition rate per day (*k*, d–1), which was used directly in the calculation of the effect size. In the few cases where litter decomposition rate was reported per degree-day (*k*, dd–1; Lecerf and Chauvet, 2008; Woodcock and Huryn, 2005), it was first converted into decomposition rate per day by multiplying by the average daily temperature over the incubation period.

The effect size of heavy metal contamination on the exponential litter decomposition rate per day was calculated as Hedges’ g, i.e. the standardized mean difference between decomposition rate in the contaminated and in the reference condition (Borenstein et al., 2009). Negative values of Hedges’ g indicate decreased decomposition rates under heavy metal contaminated conditions. For studies which reported decomposition rates at ≤ 3 levels of heavy metal contamination, Hedges’ g was calculated directly as a standardized difference between decomposition rate at each contaminated condition and reference condition. For studies that reported gradients of heavy metal contamination with > 3 levels (e.g. Fernandes et al., 2009; Medeiros et al., 2010; Niyogi et al., 2013), correlation coefficients (r) between metal concentration and exponential litter decomposition rate per day were calculated first to reduce the number of multiple comparisons per study; correlation coefficients (irrespective of significance) and associated variance were then converted into Cohen’s d and associated variance, respectively, and these into Hedges’ g and associated variance, respectively (Borenstein et al., 2009; **Table S1**). The effect of the estimation method for the Hedges’ g (i.e. directly or indirectly via r) on the results was assessed by sensitivity analyses.

The variance associated with Hedges’ g (Vg) was calculated from the standard deviation (SD) and sample size (n) associated with each decomposition rate value (Borenstein et al., 2009). If variance in the primary studies was reported as standard error (SE or 95% CL), it was converted into SD. In cases where no measure of variance associated with decomposition rates was given in the primary studies or provided by the authors, SD values were estimated by imputation based on the cases in the database that reported SD values associated with decomposition rates (Lajeunesse, 2013).

Many primary studies contributed several effect sizes to the database, for example for different litter species (e.g. Niyogi et al., 2013) or metals (e.g. Medeiros et al., 2010) (**Table S1**). Although several cases derived from the same study may be non-independent, their omission from this review would have restricted our analysis of moderators. We have therefore included multiple cases per study in the analysis, but assessed their effect on the results by sensitivity analyses. The study Pu et al. (2014) contributed a large number of effect sizes to the laboratory dataset (23%) and thus its effect on the results was assessed by sensitivity analyses.

*Moderator variables*

Several biotic and abiotic explanatory variables, referred to as moderators in meta-analysis, may affect the magnitude of the response of litter decomposition rate to heavy metal contamination. These include type of study (laboratory vs. field), type of field study (manipulative vs. correlative), identity of metal (for laboratory studies; several), origin of metal contamination (for field correlative studies; several), type of mine (for metal mines; several), pH (for coal mines; acidic vs. circumneutral), litter type (leaves vs. wood) and identity (several genera), type of decomposing community (for metal mines; microbial vs. total, i.e. microbial plus invertebrate) (see **Table S2** for the description of moderators and levels). Information on moderators was extracted from primary studies or provided by the authors (**Table S1**).

*Statistical analyses*

All statistical analyses were performed in RStudio (RStudio, 2012) with the metafor package (Viechtbauer, 2010).

Overall effect size

A random-effects model of meta-analysis (method: restricted maximum likelihood, REML) was used to determine the grand mean, i.e. the overall effect of heavy metal contamination on litter decomposition. The random-effects model was selected because there were differences in environmental conditions and methodological approaches between studies, and thus an extra source of variability, i.e. between-studies variability, has to be accounted for in addition to within-study variance. In this analysis, individual effect sizes were weighted by the reciprocal of their variance to account for differences in accuracy among studies. The mean effect size was considered as significantly different from zero if its 95% CL did not include zero. To aid in the interpretation of results, the magnitude of the effect size was considered small if ~ ⎪0.2⎪, medium if ~ ⎪0.5⎪, and large if ≥ ⎪0.8⎪ (Cohen, 1988). The percentage of total variability that is due to between-study variation rather than sampling error (I2) was also calculated (Borenstein et al., 2009).

Moderator analyses

The effects of moderators on the magnitude and direction of litter decomposition response to heavy metal contamination were assessed for subsets of the database according to our questions and available sample size; only moderator levels with at least three effect sizes were compared (**Fig. 1**, **Table S1**). We used mixed-effects models to compare heterogeneity between (QB) and within moderator levels to assess the significance of each categorical moderator (Koricheva et al., 2013). Two moderator levels were significantly different if their 95% CL did not overlap. To avoid potential non-independence between moderators, their effects were tested hierarchically (**Fig. 1**). Moderator analyses were performed only when there were at least two levels with enough sample size (levels with n < 3 were not considered) and Rosenberg’s fail-safe number (see Publication bias) was above the threshold.

Publication bias

Evidence of publication bias in the overall database was assessed by the funnel plot, and the impact it might have on the overall effect size was assessed by the ‘trim and fill’ method (Jennions et al., 2013). Evidence of publication bias in the entire database and in the datasets used in the moderator analyses was assessed by the Rosenberg’s fail-safe number (Nfs); if Nfs > 5 × n + 10, n = number of effect sizes, the results can be considered robust despite the possibility for publication bias (Jennions et al., 2013).

**Results**

*Database*

The database used in this review included 133 effect sizes from 38 primary studies. The earliest study was from 1978 (Giesy, 1978), and studies have accumulated exponentially since then (rate = 0.0932, y–1; R2 = 0.98), with studies being published at a rate of 0.2 studies y–1 in the 1980s to 1.5 studies y–1 in the 2000 – 2009 period and 2.8 studies y–1 between 2010 and 2014.

Most studies (63%) addressed the effect of heavy metal contamination under field conditions while 37% did so in the laboratory (**Table S1**). Most field studies took advantage of already existing heavy metal contamination of streams (correlative studies; 92%) and only 8% experimentally manipulated heavy metal concentration in artificial channels (**Table S1**). Correlative studies differed from each other in the origin of heavy metal contamination, the type of metal mine, the pH, the type of aquatic community involved in litter decomposition, and the type and identity of litter (**Table S1**, **Fig. 1**), which allowed us to further investigate the moderators of the heavy metal effect on litter decomposition in streams. Variation in experimental conditions among laboratory studies was lower than in field studies as the former focused on the response of microbially driven leaf litter decomposition, most often of *Alnus glutinosa*, to the different concentrations of several metals (**Table S1**, **Fig. 1**), and thus only the effect of metal identity could be assessed.

*Overall effect of heavy metal contamination on litter decomposition*

The grand mean effect size was – 0.813 (95% CL = – 1.015 and – 0.610; **Fig. 2**), indicating a significantly large negative effect of heavy metal contamination on litter decomposition. The percentage of variation explained by between-studies variation was high (I2 = 83%), and is likely due to differences in experimental approaches and environmental variables between studies (see moderator analyses below). Four effect sizes were detected missing to the left of the grand mean (funnel plot), but correcting for that with trim and fill method had little effect on the new grand mean effect size (Hedges’ g = – 0.888, 95% CL = – 1.101 and – 0.675). Also, the Nfs for the entire database was 8612, which is higher than the threshold value of 675 (= 5 × 133 + 10). Thus, publication bias is not a serious problem in our database.

*Moderator analyses*

Heavy metal contamination significantly inhibited litter decomposition in both laboratory and field studies, but the magnitude of the effect was significantly stronger in the laboratory (QB = 16.178, df = 1, p < 0.0001) (**Fig. 2**). For laboratory studies, only the combination of copper (Cu) and zinc (Zn) significantly inhibited litter decomposition (**Fig. 3**).

For field studies, heavy metal contamination significantly inhibited litter decomposition only in correlative studies; however, variation in manipulative studies is high due to low sample size, and thus no significant difference was found between field study types (QB = 0.0004, df = 1, p = 0.983) (**Fig. 2**). For correlative studies, only contamination originating from coal and metal mines significantly inhibited litter decomposition; however, again, variation in studies addressing motorway contamination is high and no significant effect of origin of metal contamination was found (QB = 2.725, df = 2, p = 0.256) (**Fig. 3**).

For studies addressing coal mine contamination, inhibition of litter decomposition was not dependent on the pH (QB = 0.034, df = 1, p = 0.853) (**Fig. 4**). Coal mine contamination only significantly inhibited the decomposition of leaves, but not of wood, and differences between litter types were significant (QB = 11.889, df = 1, p < 0.001) (**Fig. 4**). There was no significant effect of litter identity (QB = 4.807, df = 5, p = 0.440), but significant inhibition of litter decomposition was found only for four out of the six plant species tested (**Fig. 4**).

For studies addressing metal mine contamination, inhibition of litter decomposition depended on the type of metal mine (QB = 7.816, df = 2, p = 0.020), with much stronger effects for arsenic (As) mines than for gold (Au) and pyrite mines (**Fig. 5**). Only litter decomposition driven by the total aquatic community (i.e. both microbes and invertebrates) was significantly inhibited by metal contamination, but sample size was low for microbially driven litter decomposition and no significant difference was found between decomposer community types (QB = 0.633, df = 1, p = 0.426) (**Fig. 5**). There was no significant effect of litter identity (QB = 0.486, df = 2, p = 0.785), but significant inhibition of litter decomposition occurred for *Acer* and *Alnus* (**Fig. 5**).

*Sensitivity analyses*

When a mean effect size per study was considered (n = 38) instead of including all individual effect sizes per study (n = 133), the grand mean effect size did not change much (Hedges’ g = – 0.836, 95% CL = – 1.131 and – 0.540), and no qualitative changes were observed in the trends compared to those found when considering the entire database (**Table S3**). This indicates that the non-independence of effect sizes in our database does not significantly affect the results.

When the analyses were done considering the dataset for which Hedges’ g values were estimated directly (n = 95), a grand mean effect size of – 0.966 (95% CL = – 1.221 and – 0.711) was found, and no qualitative changes were observed in the trends compared to those found when considering the entire database (**Table S4**). When the analyses were done considering the dataset for which Hedges’ g values were estimated indirectly (n = 38), a grand mean effect size was smaller, but still significantly negative (– 0.219; 95% CL = – 0.426 and – 0.012).

When the analyses were done excluding the study by Pu et al. (2014), which alone contributed 23% of effect sizes to the laboratory dataset, the grand mean effect size was – 0.554 (95% CL = – 0.678 and – 0.430; **Table S5**) and there was no longer difference between the effects on litter decomposition in lab and field studies (QB = 0.806, df = 1, p = 0.369; **Table S5**). In laboratory studies, litter decomposition was significantly inhibited only by Ag and Zn contamination, although no significant effect of metal identity was found (QB = 2.500, df = 4, p = 0.645; **Table S5**).

**Discussion**

Heavy metal contamination poses a serious hazard to aquatic systems, mostly in areas of past or present mining activities (Hogsden and Harding, 2012). The present meta-analysis combines the results of 38 studies and shows that litter decomposition is strongly inhibited by heavy metal contamination, but the magnitude of the effect depended on methodological and environmental characteristics of studies. Our database consisted of 133 effect sizes derived from 38 studies, but our results are not strongly affected by the non-independence of multiple effect sizes per study as indicated by sensitivity analysis. Also, our results are not significantly affected by publication bias as indicated by the trim and fill method and Rosenberg’s fail safe numbers.

*The effect of heavy metal contamination depended on study type*

As anticipated, the effects of heavy metal contamination on litter decomposition were stronger in laboratory than in field studies, which could partially be due to a better control of potential moderator variables in the laboratory (Ferreira et al., 2015; Woodward et al., 2010). Also, the net interaction effect of these confounding factors with metals can be antagonistic leading to weaker effects in field studies. Abel and Bärlocher (1984) found weaker cadmium toxicity to aquatic hyphomycetes in the presence of calcium and magnesium ions. Recent studies found that the presence of humic acids alleviates the toxicity of smaller size copper oxide nanoparticles to microbes and detritivores (Pradhan et al., 2015, 2016) and the same could occur for metallic ions. Differences in metal identity and/or concentration between laboratory and field studies could also contribute to the distinct response observed. Laboratory studies addressed only microbially mediated litter decomposition while field studies generally addressed total (microbes + invertebrate mediated) litter decomposition. Because a stronger inhibition was anticipated for total than for microbially mediated litter decomposition (see below), the stronger response in laboratory studies (where only microbially mediated litter decomposition took place) may indicate that the better control of moderator variables in these studies was likely to be more important than the type of community involved in litter decomposition in determining the effect of metal contamination. Laboratory studies undoubtedly contribute to our understanding of the mechanisms underlying the effects of heavy metal contamination on litter decomposition. However, they likely overestimate the inhibition of litter decomposition by heavy metals compared with field studies that address the effects of metal contamination under more realistic conditions (e.g. in terms of environmental conditions and interaction among aquatic organisms). Contrary to what could be expected (Ferreira et al., 2015; Woodward et al., 2010), the magnitude of the effect was similar for manipulative and correlative field studies, likely due to the high complexity already present in the artificial channels used in manipulative experiments (channels 20 – 91 m long, with pool and run areas, and colonized by periphyton, macrophytes, macroinvertebrates and fish; Giesy, 1978; Roussel et al., 2008).

*Heavy metal contamination in laboratory studies*

Laboratory studies addressed the effects of heavy metal contamination on microbially mediated litter decomposition, most commonly of alder (*Alnus glutinosa*) leaf discs. Among the metals tested, only the combination Cu+Zn significantly inhibited litter decomposition when all the laboratory studies were considered. This effect was driven by the study by Pu et al. (2014), which contributed 12 out of the 17 effect sizes for the Cu+Zn level. This study addressed the effects of Cu+Zn mixtures on the decomposition of *Pterocarya stenoptera* leaf discs, and was the study where nutrient concentrations were the highest (Pu et al., 2014). Previous studies addressing the combined effects of changes in heavy metal and phosphorus concentrations showed complex relationships between factors that ranged from no interaction (Arce Funck et al., 2013) to significant interactions, dependent on either the metal or the nutrient concentration (Clivot et al., 2014; Fernandes et al., 2009). When the study Pu et al. (2014) was excluded from the analysis, Ag and Zn significantly inhibited litter decomposition. Silver is a potent biocide (Silver, 2003) that can inhibit bacterial growth and biofilm formation at concentrations 0.075 – 0.6 mg/L (Radzig and Koksharova, 2009). Although Zn is an essential metal, in one study considered in this review the exposure to > 32.70 µg/L significantly reduced microbial litter decomposition (Duarte et al., 2004), probably due to negative effects of this metal on the structure and activity of aquatic fungi (Duarte et al., 2004, 2008, 2009; Fernandes et al., 2009; Medeiros et al., 2010). However, in most of the studies, Zn did not affect fungal biomass or diversity (Duarte et al., 2004, 2008, 2009; Fernandes et al., 2009), but the significant reduction of fungal productivity in the study of Duarte et al. (2004) under Zn exposure corroborates the negative effects found for litter decomposition. Surprisingly, cadmium (Cd) did not significantly inhibit litter decomposition, although this can likely be attributed to low sample size since the upper bound 95% CL is already 0.027 (when not considering the study Pu et al. (2014)). Cadmium can be highly toxic to aquatic organisms (Trevors et al., 1986; Wright and Welbourn, 1994) and thus, negative effects on ecosystem processes are expected to occur.

Laboratory studies also differed in other characteristics that may partially explain differences in the magnitude of the effect between metals and in the variation of the effect within each metal. Different studies, even when addressing the effect of the same metal, used different concentration ranges (e.g. < 0.1 – 96.7 μg Ag/L in Arce Funck et al. (2013) vs. 5000 and 20 000 μg Ag/L in Pradhan et al. (2011); 15 – 35 000 μg Cd/L in Batista et al. (2012) vs. 60 – 4500 μg Cd/L in Moreirinha et al. (2011)). Since metal toxicity depends on concentration, this partially explains the high variation found. In particular, the hormesis effect, a dose response phenomenon characterized by a stimulation of activity at low doses of a stressor and an inhibition at high doses that is highly generalized (Calabrese and Blain, 2011), may generate contrasting responses of litter decomposition to metal contamination (Batista et al., 2012), leading to high variation of the effects within each metal. Also, different studies were carried out at different temperatures (12ºC – 21ºC; e.g. Arce Funck et al., 2013; Batista et al., 2012; Clivot et al., 2014; Fernandes et al., 2009; Pascoal et al., 2010; Pradhan et al., 2011). Warmer temperature has been shown to increase metal toxicity for aquatic organisms (Sokolova and Lannig, 2008), including microbial decomposers (Batista et al., 2012). Thus, litter decomposition may be inhibited by metal contamination to a larger degree at higher than at lower temperature (Batista et al., 2012). Laboratory studies also differed in the nutrient concentrations used (dissolved inorganic nitrogen (NO3-N+NO2-N+NH4-N): 40 – 8530 μg/L, PO4-P: 0 – 1740 μg/L; e.g. Clivot et al., 2014; Fernandes et al., 2009; Pu et al., 2014). Nutrient availability can also affect metal toxicity, although patterns are not yet clear (Arce Funck et al., 2013; Clivot et al., 2014; Fernandes et al., 2009).

*Heavy metal contamination in field studies*

Among field correlative studies, heavy metal contamination originating from mine drainage, but not from motorways, significantly inhibited litter decomposition. The response of litter decomposition to heavy metal contamination originating from metal mines significantly differed between mine types, which may be due to the distinct identity of the dominant metal. One arsenic mine had significantly stronger negative effects on litter decomposition than Au and pyrite (Cu) mines. However, the number of mines contributing to each mine type varied between one (As: Chaffin et al. 2005; pyrite: Schultheis et al., 1997; Schultheis and Hendricks, 1999) and three (Au: Lecerf and Chauvet, 2008; Maltby and Booth, 1991; Medeiros et al., 2008), which likely vary in other environmental characteristics besides metal identity. This highlights the lack of studies addressing the effects of metal mines on stream functioning worldwide.

Surprisingly, pH did not significantly affect the response of litter decomposition to heavy metal contamination. This may be explained by a shift between stressors from dissolved metals under acidic conditions to metal hydroxide precipitates under circumneutral conditions (Hogsden and Harding, 2012). All three stressors, i.e. acidity, dissolved metals and metal hydroxide precipitates, negatively affect aquatic communities, which often translates into reduced litter decomposition rates (Cornut et al., 2012; Niyogi et al., 2001). However, when only effect sizes estimated directly as Hedges’ g from primary studies are considered, a significantly stronger effect of metal contamination is found for acidic than for circumneutral conditions, which can be explained by the stronger effect of acidity and dissolved metals on invertebrates than on microbes (Cornut et al., 2012; Niyogi et al., 2001).

As expected, the response of litter decomposition to heavy metal contamination depended on litter type, with the decomposition of leaves being significantly inhibited while that of wood was not significantly affected by heavy metal contamination. Woody substrates generally have lower nutritional quality (e.g. high toughness and carbon:nutrients ratios) than leaf litter, and biotic activity on the former is generally lower than on the latter (Arroita et al., 2012; Ferreira et al., 2006; Gulis et al., 2004). Thus, decomposition of woody substrates was less affected by a decrease in biotic colonization and activity likely resulting from metal contamination. Also, the lower importance of invertebrates in the decomposition of wood than of leaves may have made this substrate less sensitive to metal contamination. Among leaf litter, however, there was no significant effect of litter identity on the response of litter decomposition to metal contamination, despite litter genera likely differing in quality (Ostrofsky, 1997). The differences in biotic colonization and activity between different leaf litter genera are likely to be smaller than those between leaf litter and wood, and thus the response to metal contamination is less affected by leaf litter identity than by litter type. Detection of differences between leaf litter genera may also have been hindered by low sample size, and thus results need to be interpreted with caution.

Contrary to predictions, the sensitivity to heavy metal contamination was not significantly higher for total litter decomposition than for microbially mediated litter decomposition. However, a significant inhibition of litter decomposition by metal contamination was observed when this was mediated by both microbes and invertebrates but not for microbially mediated litter decomposition. The absence of a significant effect of metal contamination on microbially mediated litter decomposition in field studies addressing metal mine effects may be partially attributed to metal tolerance exhibited by some aquatic hyphomycete species (Miersch et al., 1997) and functional redundancy that allows the tolerant species to carry out processes at rates similar to those found in uncontaminated conditions (Pascoal et al., 2005). However, the sample size for microbially mediated litter decomposition in field studies is low, and it is possible that given more studies, the effect of metal contamination will become significantly negative as the upper bound 95% CL is already 0.022. The significant inhibition of total litter decomposition by metal contamination was expected since invertebrates are highly sensitive to metal contamination (Carlisle and Clements, 2005; Chaffin et al., 2005; Niyogi et al., 2001) and may also exacerbate the effects of metal contamination on the microbial community (Arce Funck et al., 2013; Batista et al., 2012; Gonçalves et al., 2011).

**Conclusions**

Overall, mine drainage inhibits leaf litter decomposition likely through negative effects on invertebrates. However, the role of metal identity, litter identity, and type of decomposer community in moderating the effect of heavy metal contamination on litter decomposition needs to be further assessed. This assessment would be most useful if done under field conditions as laboratory studies may overestimate the effect of metal contamination on litter decomposition. Understanding the role of litter identity and type of decomposer community, in particular, in moderating the response of litter decomposition to metal contamination can contribute to the development of better plans to mitigate the effects of heavy metal contamination on stream ecosystem functioning.

Also, the recent increase in the use of metallic nanomaterials and rare earth metals in medicine, electronics and cosmetics, among other uses, and their subsequent release to the environment may constitute an additional source of metal contamination to freshwaters (Gottschalk and Nowack, 2011). The effects of nanometals on litter decomposition were not addressed in this review due to the small number of studies available. However, the effects of nanometals on aquatic organisms and processes may differ from the effects of metal ions (Griffitt et al., 2008; Pradhan et al., 2011). This makes the study of the effects of these emergent contaminants on stream communities and processes urgent.

Additionally, changes in metal concentrations may interact with changes in other environmental conditions (e.g. nutrient concentration; Arce Funck et al., 2013; Clivot et al., 2014; Fernandes et al., 2009; Pu et al., 2014), making the effects of heavy metal contamination on aquatic communities and processes highly unpredictable. Thus, future research should also focus on interactive effects of multiple stressors.

**Acknowledgements**

We thank many authors for providing information that was not available in the primary studies as well as two anonymous reviewers for the comments made on an earlier version of the manuscript. This study was partially supported by the Portuguese Foundation for Science and Technology (FCT) through the strategic project UID/MAR/04292/2013 granted to MARE. Financial support given by the FCT to VF (SFRH/BPD/76482/2011, program POPH/FSE; IF/00129/2014) and SD (SFRH/BPD/47574/2008, SFRH/BPD/109842/2015) is gratefully acknowledged.

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**Supplementary material**

**Table S1.** Database used in this review.

**Table S2.** Identification, levels and description of moderator variables used in this review.

**Table S3.** Summary table for moderator analyses considering a mean effect size per study.

**Table S4.** Summary table for moderator analyses considering the dataset for which Hedges’ g values were estimated directly.

**Table S5.** Summary table for moderator analyses considering the dataset without the laboratory study Pu et al. (2014).

**Figure captions**

**Figure 1.** Structure of the database used in this review showing the number of effect sizes for each moderator level. Levels underlined were considered in a given moderator analysis; moderator analyses were performed only when there were at least two levels with enough sample size (levels with n<3 were not considered) and Rosenberg’s Nfs was above the threshold. See **Table S2** for descriptions of moderators and levels. **– 2 column fitting image**

**Figure 2.** Effect of the heavy metal contamination on litter decomposition, overall and as a function of study type (see **Table S2** for the description of moderators). The dashed line (mean effect size = 0) indicates no effect, mean effect size > 0 indicates stimulation and mean effect size < 0 indicates inhibition of litter decomposition with metal contamination. The effect of metal contamination is significant when the 95% CL does not overlap 0 (black circles). For each moderator (indicated in bold), levels with the same letter do not significantly differ in their response to metal contamination. Values in parenthesis indicate the sample size. **– single column fitting image**

**Figure 3.** Effect of the heavy metal contamination on litter decomposition as a function of contamination origin in field correlative studies and of metal identity in laboratory studies (see **Table S2** for the description of moderators). The dashed line (mean effect size = 0) indicates no effect, mean effect size > 0 indicates stimulation and mean effect size < 0 indicates inhibition of litter decomposition with metal contamination. The effect of metal contamination is significant when the 95% CL does not overlap 0 (black circles). Levels with the same letter do not significantly differ in their response to metal contamination. Values in parenthesis indicate the sample size. **– single column fitting image**

**Figure 4.** Effect of the heavy metal contamination on litter decomposition as a function of pH, type and identity of litter in coal mine studies (see **Table S2** for the description of moderators). The dashed line (mean effect size = 0) indicates no effect, mean effect size > 0 indicates stimulation and mean effect size < 0 indicates inhibition of litter decomposition with metal contamination. The effect of metal contamination is significant when the 95% CL does not overlap 0 (black circles). For each moderator (indicated in bold), levels with the same letter do not significantly differ in their response to metal contamination. Values in parenthesis indicate the sample size. **– single column fitting image**

**Figure 5.** Effect of the heavy metal contamination on litter decomposition as a function of mine type, community type and identity of litter in metal mine studies (see **Table S2** for the description of moderators). The dashed line (mean effect size = 0) indicates no effect, mean effect size > 0 indicates stimulation and mean effect size < 0 indicates inhibition of litter decomposition with metal contamination. The effect of metal contamination is significant when the 95% CL does not overlap 0 (black circles). For each moderator (indicated in bold), levels with the same letter do not significantly differ in their response to metal contamination. Values in parenthesis indicate the sample size. **– single column fitting image**