ABSTRACT
Aquatic ecosystems are under severe anthropogenic modifications. Thus, the dependent biological communities in these environments are also changed. The objective of this study was to investigate the effects of urban development in a highly impacted ecosystem. We selected 15 sampling points along the stream Ribeirão Vermelho, in which were sampled benthic macroinvertebrates and assessed the water and habitat diversity. It was found an impact gradient, with some the reference points classified as natural and others as impacted. There was a significant difference in all biological indicators used. The total number of taxa, the wealth of Diptera, Ephemeroptera, Plecoptera and Trichoptera, and the diversity indices, the Water Quality Indices, and the percentage of herbivores crushers and predators were significantly higher in points classified as natural and changed. The relative abundances of collectors, filter feeding, chironomids and parasites were significantly lower in sites classified as natural in relation to impacted ones. The metrics of the macroinvertebrate community benthic and Habitat Diversity Protocol were influenced by environmental degradation, being a useful tool for planning and development actions for the preservation of watersheds and the prioritization of high-value transmission systems for protection and rehabilitation of aquatic ecosystems.

Keywords: biomonitoring; urban stream; benthic macroinvertebrates.

RESUMO
Os ecossistemas aquáticos estão sob fortes modificações antrópicas. Assim, comunidades biológicas dependentes desses ambientes também são alteradas. O objetivo deste estudo foi investigar os efeitos do desenvolvimento urbano em um ecossistema impactado. Foram selecionadas 15 seções de amostragem ao longo do Ribeirão Vermelho e em cada uma foi amostrada a comunidade de macroinvertebrados bentônicos e avaliada a diversidade de habitat e qualidade da água. Observou-se um gradiente de impacto ao longo do ribeirão, com alguns pontos de referência classificados como naturais e outros como impactados. Houve diferença significativa em todos os indicadores biológicos utilizados. O número total de táxons, riqueza de Diptera, Ephemeroptera, Plecoptera e Trichoptera, e os índices de diversidade, de qualidade da água e porcentagem de herbívoros, trituradores e predadores foram significativamente maiores nos pontos classificados como naturais e impactados. As abundâncias relativas de coletores, filtradores, quironomídeos e parasitas foram significativamente menores em sítios classificados como naturais em relação aos impactados. Observou-se que as métricas das comunidades bentônicas de macroinvertebrados e o Protocolo de Avaliação Rápida da Diversidade de Habitat foram influenciadas pela degradação ambiental, sendo estas ferramentas úteis para ações de planejamento e desenvolvimento para a preservação de bacias hidrográficas e a priorização de sistemas de transmissão de alto valor para proteção e reabilitação de ecossistemas aquáticos.

Palavras-chave: biomonitoramento, rio urbano, macroinvertebrados bentônicos.

INTRODUCTION
Aquatic ecosystems are subject to strong modification, especially the rivers and streams that are polluting environments receivers of the atmosphere and the terrestrial environment (ALLAN, 2004; RAMACHANDRA; BHARATH; BHARATH, 2014; MENEZES et al., 2016). Environmental change encompasses many anthropogenic disturbances; land use and their interaction are expected to have the most significant effects on biodiversity.

Land use patterns in river basins influence fundamental processes over hierarchically distributed spatiotemporal scales (ALLAN, 2004).
For example, agricultural activities can provoke erosion and runoff of sediments, nutrients, and pesticides (DUPAS et al., 2015). The anthropogenic sealing of soils in urban areas causes increased runoff, channel erosion, and threatens water quality from diverse pollutants such as metals, oils, and road salts (WALSH et al., 2005; PARR et al., 2015; MENEZES et al., 2016). Riparian clearance and subsequent increased solar radiation elevate water temperature and alters fundamental physicochemical processes (BOOTH et al., 2014). Combined effects of increased concentration of contaminants, temperature elevation and hydrologic changes modify dependent communities of aquatic ecosystems (PARR et al., 2015).

Amongst the communities of aquatic ecosystem, the benthic macroinvertebrate fauna is by far the most widely studied and well known in freshwater biomonitoring studies, a result of their ease of sampling, well studies taxonomic and functional diversity, ubiquity, and comparatively rapid response to a variety of stressors and life-cycle patterns that are compatible with timescales associated with the persistence and effect of known stressors such as organic pollution (FRIBERG et al., 2011).

The bioindicators concept for assessing the quality of freshwater systems, such as rivers and streams is not new. As Birk et al. (2012) succinctly put it, "aquatic bioindicators are organisms accumulating toxic substances or responding to environmental stress, such as pollution, nutrient enrichment, habitat loss or overexploitation".

Several methods have been developed to assess the ecological status of surface waters in Brazil, among them, the use of metrics has been an alternative to national indices for assessing the effects of changes in land use. Metrics synthesise biological and ecological information of macroinvertebrate assemblages (HAYBACH et al., 2004) and are frequently used to identify change in relation to natural and anthropogenic gradients (VARANDAS & CORTES, 2010; FEIO & DOLÉDEC, 2012; TUPINAMBÁS et al., 2014). Some studies have associated changes in human disturbance with an integrated index of biotic integrity, subsampling changes in individual metrics in the larger index (GUIMARÃES et al., 2009; VARANDAS & CORTES, 2010; CORTES et al., 2011; TUPINAMBÁS et al., 2014). Other studies have demonstrated associations between stressors and particular biological metrics, but these associations were expressed only in terms of whether a statistically significant positive or negative relationship existed and, thus, did not investigate the relative responses of the metrics.

Developing biomonitoring tools requires evaluation and definition of the efficient ways to detect human impacts. In this way, the aim of this study was to evaluate how different types of land use influence the metrics of the benthic macroinvertebrate communities. It was tested the hypothesis that the benthic community using metrics will answer in differentiated way to the impacts caused for the different types of land use activities in comparison with the regions with absence of anthropogenic disturbances, in order to implement appropriate policies and management practices to minimize the impacts of urbanization on aquatic ecosystems.

**MATERIALS AND METHODS**

The methodological steps are described in the next sub-items, including the study area, physicochemical and hydrological parameters, land use, benthic macroinvertebrates, data treatment and statistical analyses.

**Study area**

Located in the south of Minas Gerais region, the Ribeirão Vermelho river basin, a tributary of the Rio Grande, crosses the city of Lavras (Figure 1). Soil management practices are poor, with 49.3% of the basin occupied by unmanaged pasture and agriculture (MENEZES et al., 2014) and 21.4% by urbanization. The region is subtropical (Köppen classification — Cwb) with an annual average temperature of 19.3°C (maximum of 21.3°C and minimum of 15.1°C).

A total of 15 sampling stations with different degrees of anthropogenic impact and potential sources of pollution (urban area, pasture and agriculture) were selected along the Ribeirão Vermelho river (Figure 1). We conducted seasonal sampling campaigns over 1 year in 2014, which were equally distributed for the rainy season period in February/May and for the dry season period in September/October (2 surveys in each period).

**Physicochemical and hydrological parameters**

Electrical conductivity, pH, temperature and turbidity were measured in situ using a multiparameter probe (HI 98280, Hanna®). The following physicochemical and biological parameters were analysed in the lab following Standard methods for the examination of water and wastewater (APHA, 2007) protocols: chloride (Cl; mg.L⁻¹), electrical conductivity (EC; µS.cm⁻¹), biochemical oxygen demand (BOD₅; mg.L⁻¹), phosphate (PO₄³⁻; mg.L⁻¹), nitrate (NO₃⁻; N; mg.L⁻¹), dissolved oxygen (DO; mg.L⁻¹), total solids (TS; mg.L⁻¹), dissolved solids (DS; mg.L⁻¹) temperature (T; °C), turbidity (Tur.; NTU) and thermotolerant coliforms (TC, MPN 100 mL⁻¹). Water velocity was measured with a jctm-hidromet® portable flow meter (four measurements per site). Inorganic substrate was collected at each site with a corer (a composite sample comprising four cores per site) to approximately 10 cm depth. The granulometric composition of the collected substrate (%) was determined after drying by sieving through a set of Unite States Standard sieves that separated the material into pebble, gravel, coarse sand, fine sand, and silt/clay fractions (BLOTT & PYE, 2001). Stones and boulders were not observed in the study area. Organic content (%) was determined using the gravimetric ash-free dry weight method. Aliquots (0.3±0.1 g) were ashed (550°C for 4 h) and weighed; the difference between the initial weight of sample and weight after ashing gave the percentage of content of organic sediment samples.
Land-use
Land-use data were obtained from Google Earth satellite images. Screen scanning of features was made at the scale of 1:1,500 (ArcGIS 10, ESRI enterprise) using image interpretation techniques (supervised classification) to obtain information of proximal land use type, percent occupation and distribution at each sample station (SANTOS; LOUZADA; EUGÊNIO, 2007). A land use classification scheme (agriculture, forest, pasture, soil, urban area and water body) was developed based on current land use condition classification for Lavras (MENEZES et al., 2014), based on IBGE (2006) methodology and Santos, Louzada and Eugênio (2007). Reach level data were obtained by defining a 250 m radius buffer around each site based on the findings of Cortes et al. (2011) on the association of aquatic communities and environmental descriptors at different spatial scales.

In each study site, the Rapid Assessment Protocol (RAP) for habitat diversity (HANNAFORD; BARBOUR; RESH, 1997) modified by Callisto et al. (2002) was used to analyze 15 parameters (see CALLISTO et al., 2002). In the first group, the score assigned to each parameter was 0, 2 and 4, and in the second, 0, 2, 3 or 5 points. The final result was the sum of the scores assigned to each parameter. Final scores indicate the level of ecological preservation of the sites under study, scores from 0 to 20 indicate that the sites have been poor (impacted); from 42 to 60, have been altered or suboptimal; and from 61 to 88, are in optimal condition (natural).

Benthic macroinvertebrates
Three replicate samples of benthic macroinvertebrates were taken in riffle/run sections at each sampling site (total of 180 sub-samples) on each sampling occasion (pools comprised less than 10% of total stream channel area at all sites) using a Surber sampler (250 μm mesh, 0.1 m²). Organisms were preserved in 70% ethanol, and then identified to family level using specialized literature (TACHET et al., 2000; MUGNAI; NESSIMIAN; BAPTISTA, 2010).

Data treatment and statistical analyses
Redundant modalities and environmental data were removed using Spearman Rank Correlation (threshold value r ≥ 0.65) and draftsman plots (HUGHES et al., 2009) to avoid multicollinearity.

Figure 1 – Location of the 15 sampling stations along the Ribeirão Vermelho river, in the Ribeirão Vermelho basin, in southeast Brazil.
We derived three biotic data sets to compare the effect of ecological conditions, water quality, habitat diversity and land-use on taxonomic composition (family level identification, relative abundance log10 \((x+1)\) transformed), metrics describing community structure and composition (standardized). A Principal Components Analysis (PCA) was carried out on environmental data to reduce dimensionality, identify principal environmental gradients and construct orthogonal stressor gradients.

A total of 16 metrics describing community composition and structure were calculated using the ASTERICS software, version 3.3.1 (AQEM Assessment System, Essen, Germany), including richness measures: number of taxa \((s)\), numbers of families, abundance of macroinvertebrate \((N)\), percentage of Ephemeroptera \((nEphemeroptera)\), Plecoptera \((nPlecoptera)\) and Trichoptera \((nTrichoptera)\), EPT richness, percentage of Oligochaeta, Hirudinea, Diptera \((nOligochaeta, nHirudinea\) and \(nDiptera)\). It was also calculated the Shannon–Wiener Index \((H')\), Margaref index and Simpson index. They are also selected biological indexes, Biological Monitoring Working Party (BMWP) Score and Danish Stream Fauna Index (DSFI) Diversity Groups.

Pearson’s product-moment correlation coefficient \((r)\) was used to analyze relationships between metrics and land use values. All analyses were carried out using PRIMER 6 software (PRIMER-E Ltd) (CLARKE & GORLEY, CLARKE, 2006), PERMANOVA + for PRIMER software (ANDERSON; GORLEY, CLARKE, 2008) and Statistica 10.0 (STATSOFT, 2011).

RESULTS AND DISCUSSION
The protocol for characterization of ecological conditions indicated advanced environmental degradation in the Ribeirão Vermelho, with impacts on habitat diversity, bank erosion, clearance of riparian vegetation, riverbed instability and water body siltation. Upstream sampling stations (stations 1–3), situated in sections where springs, protected areas and riparian vegetation are present, were classified as natural (scores ranging from 71 to 90). Sampling stations 5–7, 14 and 15 were classified as altered (scores between 44 and 51), and sampling stations 4, 8 to 13 were classified as impacted (scores between 12 and 40).

Using the Protocol Rapid Assessment proposed by Callisto et al. (2002), the ecological conditions and habitat diversity in the catchment stretches showed that, because it is an urban stream, the human influence has become an important factor for low maintenance levels of ecological conditions found in most stations collecting along the Ribeirão Vermelho river. The station next to the nascent of the Ribeirão Vermelho is found in natural condition of preservation, allowing the natural development of the macroinvertebrate community. This pristine stretch and its surroundings provides greater source of organic resources and stability of the stream bed allowing greater habitat heterogeneity and substrate to be colonized by aquatic macroinvertebrates (CUMMINS & KLUG, 1979).

Excerpts from the watercourse that were considered to have altered next score impacted condition, showing that changes are taking place around the Ribeirão tending to homogeneity of habitats. According GALDEAN et al. (2000), evaluation of habitat diversity is an important tool in health approach to aquatic ecosystems due to the strong relationship between availability of habitats and aquatic diversity. Although protocols based on visual qualifications are simple application tools, allowing an immediate diagnosis of the state of the body of water, they must have their interpretation always linked to other studies that are designed concomitantly (CORGOSINHO et al., 2004). The combination of information from the physical characterization with the analysis of water quality provides evidence of the ability of a stream to support a healthy aquatic community and the presence of chemical and organic pollution in the aquatic ecosystem (TUPINAMBÁS et al., 2014).

The PCA plot (Figure 2) revealed a clear quality gradient along the first axis. “Natural” sites were distinct from remaining sites. The first two PCA axes explained 45.8% of the total variance based on selected environmental parameters (Figure 2). PCA1 explained 28% of the total variance with positive loadings for DO \((0.37)\), Forest \((0.27)\) and Soil \((0.05)\) and negative loadings for BOD \(_3\) \((-0.39)\), Log DS \((-0.38)\), Turb. \((-0.33)\), EC \((-0.32)\), Urban \((-0.25)\) and NO\(_3\) \((-0.17)\).

“Natural” sampling stations were strongly correlated with DO results and forest and agriculture (i.e., non-urban) land-use categories (Figure 2). “Altered” sampling stations were situated in tributaries and the final downstream sampling site, characterized by bank erosion and channel siltation. Water quality parameters indicated organic enrichment \((\text{NO}_3, \text{BOD}_3)\) and land use was predominantly urban. “Impacted” sampling stations, located within the city of Lavras, typically had no

Figure 2 – Principal components analysis (PCA) of the Ribeirão Vermelho sampling sites based on environmental and habitat diversity protocol.
vegetation, reinforced river channels, high levels of organic pollution (NO$_3^-$, Log DS, EC and BOD,) and urban.

Urban streams are subject to a “cocktail” of anthropogenic stressors, impacts and contaminants. Macroinvertebrate assemblages respond to combinations of environmental (e.g., abiotic factors such as flow and substratum) and pressure drivers that may act together or in isolation (WIBERG-LARSEN et al., 2000). The “habitat template” theory proposes that temporal and spatial variability in physical habitats influence the evolution of physical, behavioural, and physiological species profiles (SOUTHWOOD, 1977). The results of this study on a partly urban stream show shifts in macroinvertebrate assemblage structure expressed via taxonomic groups and macroinvertebrate metrics response to urban impacts.

According to Ormerod et al. (2010), in freshwater management, these problems almost always involve simultaneous challenges because human pressure usually change more than an environmental factor (for example, urbanization affects the amount of flow, water quality, habitat availability and others), making it difficult to establish direct associations between drivers and change in assemblages (STEWART et al., 2001). Results from our study on macroinvertebrate assemblage structure and function support this statement.

A total of 54 taxa and 68,808 individuals were identified and counted in the study. Mean total density was a lower at most “natural” site than at “altered and impacted sites”. The Ribeirão Vermelho river presented wealth of coincident taxa with those observed by other authors in streams with similar anthropogenic influences. Among them are the works of Moreyra and Padovesi-Fonseca (2015) — 44 families, Dos Anjos Santos et al. (2016) — 41 families, and Docile et al. (2016) — 35 families. Importantly, these studies were performed with different methods and geographic areas, which makes faunal comparisons. At altered and impacted sites, benthic Chironomidae, Psychodidae, Hirudinea and Oligochaeta dominated communities. Plecoperata were most abundant at natural sites, whereas Diptera and Annelida were most abundant at urban sites (altered and impacted sites).

The results of the distribution of fauna show that the type of use or land cover in each section appears to provide specific conditions that determine the appearance of different biotypes. This community at a certain place reflects all factors involved in their development. Thus, a balance in the ecosystem biotic conditions is diverse and develops complex relationships between organisms and the environment, allowing these communities remain stable. If local conditions change, it will be immediately in the medium or long-term physical and chemical changes of water and therefore the disruption of the existing balance. The changes that follow the structure of aquatic community’s alternate are complex and diverse with own clean water bodies, simple and low diversity, with their own bodies polluted water, easily varying with the effect of pollution (MOREYRA & PADOVESI-FONSECA, 2015).

Modifications to the structure and composition of macroinvertebrate community can occur due to changes in the viability of trophic resources (TUPINAMBAS et al., 2014). Selected metrics were able to discriminate different degrees of biotic integrity. Species richness, EPT richness and Diversity Index (Shannon-Wiener and Simpson) were lower at all altered and impacted sites than natural sites (Figure 3). All impacted sites had lower BMWP and DSFI values than their respective natural sites. The most dramatic changes were recorded at impacted sites. Percentage of Hirudinea and Oligochaeta density did not differ between natural and impacted sites, but at altered site Hirudinea was different, show more number of individuals. Percentage of Diptera increased from natural to impacted and altered sites, and was most pronounced in altered sites, whereas the percentages of Ephemeroptera, Plecoptera and Trichoptera was higher at natural sites than at altered-impacted sites in Ribeirão Vermelho stream (Figure 4).

Macroinvertebrate density patterns varied considerably among natural and impacted-altered sites. Thus, mean macroinvertebrates density was higher at impacted sites. In this study, they were selected several metrics commonly used to quantify the ecological degradation in general and organic contamination. In many cases organism groups and metrics responded predominantly to specific stressors. Our results indicate that metrics based on macroinvertebrate communities were useful to identify different degrees of pollution and disturbance in the studied urban streams. The impacted-altered site was shown to be the most disturbed reach by the majority of metrics. The results suggest that the ratios tested produce very consistent results with each other, and similarly broadly reflect variations in the structure of the communities and their sensitivity to natural environmental factors.

It was expected an improvement in water quality owing to presence of the wastewater treatment plant at Lavras, but none of the physical-chemical and biological parameters suggested stream water recovery. However, in the last year, Lavras has experienced a growth in population, and the ability of the plant to cope with the volume of waste produced now appears to be inadequate. Also, the impervious surfaces of the town have increased as more streets and neighborhood areas have been paved. In some areas, the domestic sewage system is frequently flooded by storm-water, thus sewage and pluvial drainage systems are not always working as separate units high discharge events associated with rains. This result is a serious alarm for local managers to consider stricter control measures on the aquaculture activities.

A reduction in taxon and EPT richness and an absence of some taxa with low tolerance to sedimentation were reported in an urban river in northern Rio Grande do Sul, Southern Brazil (HEPP & SANTOS, 2009), while in some rivers, the occurrence of EPT taxa seems to be affected by factors associated with riparian cover and catchment land use (BERTASO et al., 2015). There are several factors that can influence
Figure 3 – Distribution of values of selected metrics for macroinvertebrates at natural, impacted and altered sites on the Ribeirão Vermelho river. Range bars show maxima and minima, boxes are interquartile ranges (25–75%), small squares are medians.
Figure 4 - Distribution of values of selected metrics for macroinvertebrates at natural, impacted and altered sites on the Ribeirão Vermelho river. Range bars show maxima and minima, boxes are interquartile ranges (25–75%), small squares are medians.
the structure of community’s benthic macroinvertebrates at different scales. For example, factors abiotic as speed and water depth, type of substrate, and matter organic influence the distribution of macroinvertebrados scale microhabitat (ALLAN, 2004), while hydromorphological, thermal conditions or geomorphological tend to affect the distribution and structure of communities the longitudinal river gradient (VANNOTE et al., 1980). Also, factors biotic, such as predation and competition, can influence the structure location of invertebrate communities (ALLAN, 2004).

Except for percentage nPlecoptera and nDiptera, all metrics showed at least one significant correlation with land use variables (Table 1). The Diversity index (Shannon–Weaver, Margaref and Simpson), nEphemeroptera, nPlecoptera, EPT, number of taxa (s) and number of families showed positive and significant relationships with forest areas and agriculture areas, all of these metrics are related to good ecological integrity. In contrast, these rates had a negative correlation with urban areas. Urban areas showed positive and significant relationships with nOligochaeta, nHirudinea and abundance of macroinvertebrate (N).

Benthic invertebrate metrics and biotic indices differed between types of land use, which indicates an increasing gradient of impairment from upstream to downstream Ribeirão Vermelho river. High on the watershed of the Ribeirão Vermelho is still possible to find green areas, while in the downstream direction the stream goes through city of Lavras to the following section for agricultural areas (Figure 1). The degree to which current assemblage structure in these streams reflects land-use history is unknown. In summary, the modification land use gradient was reflected in the changes in the benthic macroinvertebrate communities, which indicated ecological impairment at structural level.

Previous studies have shown that sensitive species were absent or less abundant in streams draining urban areas (WALSH et al., 2005; JOHNSON et al., 2006; ORMEROD et al., 2010; FEIO; DOLÉDEC; GRAÇA, 2015; PARR et al., 2015). Overall, the flows in urban areas are characterized by clusters of species of the poor, consisting mainly of disturbance tolerant rate. Sets of highly degraded streams in urban watersheds are numerically dominated by some species of Oligochaeta, Hirudinea and Diptera (WALKER et al., 2009). In a recent study, Wang et al. (2012) showed most of the macroinvertebrate indices, including total taxa richness, EPT taxa richness, Diptera taxa richness, and percentages in abundance of shredders, filterers, scrapers, and collectors differed significantly among forested stream sites, agricultural sites, and urbanized sites.

**CONCLUSIONS**

In conclusion, a trend in the benthic community observed in predicting changes and alteration of habitat quality caused by different land uses and alteration of habitat quality, especially when the source point pollution, such as the case of the urban area. Sampling points located near the urban area showed significant changes in macroinvertebrate density due to predominance tolerant species.

The response of the taxonomic composition and metrics of benthic macroinvertebrates assemblages to different stressors varied in the basin of the Ribeirão Vermelho stream. Land use and parameters of water quality were important variables that affect the answers of the data groups (taxonomic composition and metrics) of macroinvertebrates.

It was proposed to implement an integrated approach in the assessment of the health of urban aquatic ecosystems involving the analysis of the processes at the ecosystem level, as well as structural and functional biological indicators. These results are important for local managers of the studied river as well as those of other rivers in Minas Gerais, which are under stress of the same land uses.

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