INTEGRATED APPROACH FOR THE ANALYSIS OF ECOSYSTEMS AT RISK: A CASE STUDY IN A WASTE LANDFILL

Extended abstract

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Background

Waste landfills are currently the most widely used method to dispose municipal waste, mainly in highly populated cities. Landfills can be a source of a large number of toxicant elements and compounds diffuse through air fallout or leachate release. Some research has been shown that landfills spreads a large number of compounds that, since industrial revolution, are increasingly accumulated in the soil and in the litter and that in some situations, may be available for the species even at considerable distances from the source (Gagnon and Saulnier, 2003).

There are many studies on the chemical characterization of leachate and air but they represent only the first step for a meaningful environmental impact evaluation. For these reasons one of the most important activities concerning the management of a municipal waste landfill regards the control and environmental monitoring aimed to safeguard both of the structural aspects of the plant (i.e., efficiency) both the environmental quality of the site.

Quantification of heavy elements levels in soil and air cannot generate sufficient information on impact because the absolute metal concentration alone does not reflect the degree to which these compounds affect the environment (Cheung et al., 1993). Data on the bioaccumulation and effects of leachate on wild populations are essential to assess the environmental impact of these disposal sites.

In this paper we propose an evaluation method that brings together biomonitoring techniques, morphometric measurements (fluctuating asymmetry), cytometric tests on cells of the hepatopancreas of Armadillidium vulgare (Isopoda, Crustacea, Latreille, 1804) and quantitative analysis of lichens; an integrated approach that can provide information about the state of health of the ecosystem before it gets evident alterations

Objectives

- Enlarge the field of study of the monitoring compartment by inserting the "organic".
- Detect contamination so early before if their effects occur through the use of soil macro-invertebrates and lichens.

Outline of the work

This work is divided into four parts:
- Analysis of heavy element contents in terrestrial isopods (Armadillidium vulgare)
- Analysis of heavy element contents in lichen transplants
- Ratings of fluctuating asymmetry in Armadillidium vulgare
  Flow cytometric analysis of hepatopancreatic cells of Armadillidium vulgare

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Methods

The primary study area is centered on the municipal solid waste landfill of Ginestreto and extends on a surface of about 25 km² in the municipality of Sogliano al Rubicone (Emilia-Romagna, Italy). In order to evaluate the impact of the landfill fallout on the surface environment, epiphytic lichens and terrestrial isopods were used as bioindicators of air and soil contamination. Leachate employed in cytometric laboratory tests has been collect from the same site. Only for asymmetry study isopods were collected isopods in other two sites as control/comparison. The first is located at the campus of Urbino University (Marche, Italy), a protected woodland called "Fosso del tasso" in which there are no significant human activities. The second is in the area of Pantiera (Urbino, Italy in an agricultural context.

Lichens

Lichens are organisms commonly used in air monitoring studies as they are highly efficient accumulators of many chemicals such as heavy elements. Due to this ability, it has been assumed that the heavy element concentrations in lichens reflect their atmospheric levels. Consequently, these organisms are considered important biomonitors of air quality and are extensively employed to assess the impact of several human activities (e.g., vehicular traffic, industrial and power plants, incinerators and smelters) on atmospheric contamination. Lichen transplants are frequently employed in air monitoring surveys (e.g., Ayrault et al., 2007). The advantages of lichen transplants are: uniformity of species, entrapment surface and exposure period, flexibility in selection and number of monitoring sites, knowledge of the original concentrations of contaminants in lichens before exposure, opportunity to carry out biomonitoring studies in areas where native lichens are absent.

In this study the transplanted lichens were collected in an uncontaminated area of central Italy. Before exposure, thalli were washed to remove the particles trapped on the surface and acclimated for 24 h in a cell conditioned at a temperature of 15±2°C. The lichen transplants were exposed for 4 months (October 2012 – January 2013) in 31 sites located: i) close to the municipal solid waste landfill; ii) close to the main roads of the study area; iii) in zones far away from the potential anthropogenic sources of contamination. After exposure, the lichens were frozen at -20°C and pulverized with liquid nitrogen. The pulverized lichen samples were solubilized by acid digestion. The digestion was performed in a microwave lab station using a mixture of ultrapure reagents (HF, HNO₃ and H₂O₂). The solution was analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) to determine the concentrations of As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Sb, Tl and Zn.

Isopods

The terrestrial isopods are considered bioindicators of soil quality and suitable for studies on environmental contamination. For their important role in decomposition processes in surface soil, these invertebrates are in close contact with soil contaminants and are able to accumulate heavy elements. Adult individuals of Armadillidium vulgare isopod were collected by hand-sorting in June 2012. The isopods were sampled in 13 sites placed at different distances from the waste landfill (up to 8 km) along a transect aligned to the main wind direction. In each sampling site 10 isopod individuals were collected within a range of about 100 m.

Heavy element analysis

In laboratory isopods were rinsed with deionized water to clean them of adhering soil particles; then they were kept for 72 h in plastic Petri dishes at a constant temperature of 18°C with a photoperiod of 12 hours, to allow emptying of the intestinal tract. The isopod specimens were killed by freezing at -80°C and freeze-dried for 48 h. The freeze-dried isopods were digested with a mixture of ultrapure reagents (HNO₃ and H₂O₂) in a microwave lab station. The solution was analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) to determine the concentrations of As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Sb, Tl and Zn.

Fluctuating Asymmetry

Fluctuating asymmetry (FA) is the random and non-systematic deviation from the normal condition of symmetry, occurring without stressing factors, be they environmental or genetic. Our hypothesis is that the FA will be greater in the presence of environmental stresses such as pollution. We compared the morphological alterations of three pools of isopods sampled in rich habitats with different land use and in general with a different anthropogenic pressure. In particular an optical microscope associated with a camera was used to capture photographs of seven symmetrical traits: number of composite ommatidia of the eye, length of the first flagellum, second and the third segment of the antenna, merus, carpus and propodus of the seventh pareiopods. The captured images of nearly 100 specimens were processed by the image analysis software Image J. The asymmetry index used is defined as (Godet et al., 2012)(Eq.1):

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FA = \frac{|R - L|}{R + L}/2
\]

where: FA is fluctuating asymmetry; R is right size; L is left size.
Flow cytometry of hepatocytes

The hepatopancreas of the terrestrial isopod *Armadillidium vulgare* takes an important role in bioaccumulation of contaminants, such as heavy elements. To evaluate the effects of landfill leachate treatment Flow Cytometric techniques were, for the first time, applied to the hepatopancreatic cells of isopods. This organ (*Armadillidium vulgare*) has been thoroughly investigated by many authors, due to both their morphologic characteristics, which are related to the physiological condition, and their role in bioaccumulation of contaminants. The terrestrial isopods hepatopancreas is composed of four blind-ending tubes. The hepatopancreatic epithelium contains two cell types: the large B cells and the wedge-shaped S cells that lie between the B cell. The main features of B cells are the presence of well developed microvilli, active Golgi complexes, extensive arrays of rough endoplasmic reticulum, numerous mitochondria and a lot of lipid droplets. This ultra-structural features suggest the role of the B-cells in secretion of digestive enzymes and absorption of nutrients. The S-cells accumulate large amounts of elements such as calcium, and uric acid salts. Experiments of contamination of isopods with landfill leachate were conducted in order to verify the different behaviour of hepatopancreatic cells during 30 days of exposition (Manti et al., 2013).

![Fig. 1. Normal appearance of digestive epithelium of external gland (a); normal appearance of digestive epithelium of internal gland showing B and S cells (b, c). Electron microscopic analyses (ESEM) show the morphologic characteristics in hepatopancreas of *Armadillidium vulgare*, in particular the images underline the different size of cells (ranging from 20-30 µm and 50-70 µm Ø respectively).](image-url)

**Results and discussion**

**Lichens**

The analytical data indicated that in the monitoring sites of the study area the heavy element concentrations in transplants are usually higher than their levels in non-exposed lichens. According to the *Exposed to Control ratio* (EC ratio = element concentration in lichen transplant / element concentration in non-exposed lichen) Cd, Cr, Pb, Sb and Zn are in severe accumulation in the transplants placed close to the municipal waste landfill of Ginestreto as well as in some sites close to the main roads. In the landfill sites these heavy elements reached the highest enrichments with EC ratio average values of 4.7 for Sb and about 2 for Cr, Pb, Sb and Zn. As, Co, Cu, Ni and Tl are accumulated by lichens with EC ratios usually within 1.25 and 1.75. No accumulation process was found for Hg.

**Heavy element accumulation in Isopods**

The heavy element concentrations in isopods were rather uniform. Cd, Pb and Sb showed the highest contents in individuals collected in the traffic-influenced sites. Among the analyzed heavy elements, Cd, Cu and Zn were bio-accumulated by isopods with average values of the Bio Accumulation Factor (BAF = element concentration in isopod / element content in soil) of 1.1, 6.1 and 1.3, respectively. These results are in agreement with data reported by other authors (Blanusa et al., 2002) as well as in our research realized in a similar environmental setting.

**Fluctuating Asymmetry**

Performing a test of the effects between subjects and a comparison in pairs on the three sampling sites, we obtained that the most significant differences are for the traits metric unlike those meristic (number of ommatidia) that are not significantly different. We also verified that the index of F.A. shows the most significant differences between the landfill site and the control group.

**FCM analysis**

In this study, we correlated the presence of Big and Small cells, in terms of cell count and viability, with the presence of some elements. Our findings were underlining Small SYBR Green I (a fluorochrome able to labelled nucleic acids) Bright cells correlate with specific chemicals (Ca, Cu, Co) confirming their resistance to stress stimuli.
Furthermore, we also investigated B cell trends that highlight a general 2 fold increase in Propidium Iodide positive frequency in respect of S cells, confirming their more pronounced sensitivity to environmental stress. In samples observed the S cells are more abundant than B cells. Substantial differences in metal accumulation are known between the isopod species Porcellio scaber. Considering that B cells seem to play a role in metal excretion and S cells in metal storage we may assume in Armadillidium a clear prevalence of S cells.

Fig. 2. Cytometric dot plot FSC vs. SSC of hepatopancreatic cells

Concluding remarks

Soil is a dynamic system and it is subject to dilution phenomena, erosion and transport of materials and pollutants. Air and soil chemicals analysis can determine an underestimation of the environmental impact, because this last can manifest itself as ecological debt. The use of biological indicators can be integrated to traditional monitoring to give a qualitative and quantitative evaluation of the impact on biological systems. We can conclude that isopods and lichens are efficient bioindicators also for landfill fallout, and their response to the pollutant effects can be consider as a good marker to evaluate the real ecological damage of a contaminated environment.

Keywords: flow cytometry, isopods, leachate, lichen transplants, waste landfill

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