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Haematological and immunological response of *Achyranthes aspera* leaf and root extracts in arsenic-intoxicated female mice (*Mus musculus*)

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To evaluate therapeutic efficacy of *Achyranthes aspera* against arsenic toxicity, mice were given aqueous root and leaf extracts at both low and high doses (100 and 200 mg/kg body wt) after being intoxicated with sodium arsenate (0.1 mg/kg body wt). Significant alterations ($P < 0.05, 0.001$) were seen in various haematological parameters, Ig level, macrophage yield, viability, phagocytic index and progesterone level. Results clearly depict that both *A. aspera* extracts significantly restore the unbalanced level up to the normal. This study shows the protective efficacy of *A. aspera* on altered haematological and immunological system. It is possible that future work on drug formulation may use this plant as a source.

Keywords: *Achyranthes aspera*, arsenic toxicity, haematological alterations, mice immune system.

ARSENIC (As), is present in various forms in soils, pesticides, groundwater, drinking water, rocks and fossil fuels[1]. Presently, due to enhanced human activities like mining, smelting, coal combustion, etc., the level of arsenic has crossed its permissible limit and comes in contact with humans via various routes – inhalation, ingestion and dermal absorption. Inorganic As reacts with –SH group of cell proteins and inhibits various oxidative processes, thus leading to various health problems[2–4] like tissue hypoxia, cell damage, hepatic and central nervous system damage[5–8]. To combat As toxicity, a range of therapies are available that are based on chelation of As from the body via various synthetic agents that lead to severe side effects in the body. So, there is a need to develop a potent herbal drug that has fewer side effects and is more target specific.

Achyranthes aspera (family Amaranthaceae), a perennial stiff erect herb has cosmopolitan distribution. This plant is traditionally used in the treatment of various diseases like odontalogic, rheumatism, bronchitis, skin diseases, rabies[9], fever, dysentery and diabetes. The plant also works as an antiviral, anticoagulant, antihypertensive, diuretic, aphrodisiac, antifertility, antispasmodic and antitumour[10–12]. It has been reported that the leaf and root parts of *A. aspera* contain ecdysterone (phytoecdysone), oleanolic acid and other important bioactive constituents like flavonoids, saponins, alkaloids, glycosides, etc.[13]. Hence, recognizing the toxic effects of arsenic and the therapeutic efficacy of *A. aspera*, the present study was designed to evaluate the haematological obliterations and immune-modulating effect of *A. aspera* in sodium arsenate-intoxicated mice.

All chemicals used in the study were of analytical grade and were purchased from reliable firms (Sigma-Aldrich, SRL, Merck-Millipore, RanBaxy and HiMedia). Sodium arsenate, the experimental compound, was purchased from HiMedia, India.

A. aspera was collected from the roadside at the Banasthali University Campus, Rajasthan, India and was taxonomically identified by a botanist of Krishi Vigyan Kendra, Banasthali University. Root and leaf parts were separated from the whole plant, cleaned, shade-dried and powdered. Aqueous extracts of both parts were prepared by simple maceration method. The filtrates were concentrated under reduced pressure in a rotary evaporator (Heidolph Incarp Instruments Pvt Ltd, Germany) and stored at room temperature in desiccators for further analysis.

Female Swiss albino mice (weighing 20–30 g) were obtained from Haryana Agricultural University, Hissar, India for experimental purpose. The Animal Ethical Committee of Banasthali University approved the experimental protocol. All animals were housed in polypropylene cages under well-maintained temperature (25 ± 3°C) with 12 h alternating light and dark cycle. Mice were provided nutritionally adequate pelleted chow diet (Ashirwad Pvt Ltd, India) and drinking water *ad libitum* throughout the study.

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Table 1. Grouping and treatment schedule of mice in the experimental regimen

Group number	$n = 8$ in each group	Treatment
1	Control vehicle only	Distilled water
2	SA	0.1 mg/kg body wt orally for alternate 7 days
2a	SA + AALE-L	*Achyranthes aspera* leaf extract low dose; 100 mg/kg body wt
2b	SA + AALE-H	*A. aspera* leaf extract high dose; 200 mg/kg body wt
2c	SA + AARE-L	*A. aspera* root extract low dose; 100 mg/kg body wt
2d	SA + AARE-H	*A. aspera* root extract high dose; 200 mg/kg body wt

SA, Sodium arsenate.

A total of 48 adult female mice were divided into 6 groups ($n = 8$ mice). Table 1 shows the experimental regimen of the study.

All doses, including the toxic compound and *A. aspera* extracts were administered orally in mice. The designed study was post-treatment. Plant doses were started on the same seventh day after 2 h of administration of SA in mice for 15 days regularly with total treatment duration of 22 days. Dose for SA was decided on the basis of Material Safety Data Sheet-7170-1 and the concentration of SA used in the experiment was 1/10 of LD_{50}. Plant dose was decided on the basis of previously published reports[14] and also on the experiments conducted in our laboratory. For study of immunological parameters mice were antigenically challenged with sheep RBCs, with first challenge on the second day and second on the fifth day of the experiment. After complete treatment, mice were fasted overnight; the next day they were sacrificed by cervical dislocation and blood was collected by cardiac puncture in EDTA-coated vials for haematological assays and also in simple eppendorf tubes to collect serum. Clear yellowish serum was collected after the clotted blood was centrifuged at 5000 rpm, 4°C for 15 min.

Peritoneal fluids of all treated groups were collected in centrifuge tubes by injecting ice-cold saline (0.9%) in peritoneal cavity of mice. Cell suspension was then centrifuged at 1000 rpm for 10 min at 4°C to obtain the pellets, then washed with 5 ml of chilled saline twice and centrifuged. Pelleted cells were re-suspended in 0.5 ml of ice-cold saline.

Various haematological parameters like total erythrocyte count (TEC), haematocrit value (HCT), total WBCs count (TWC), haemoglobin (Hb), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), platelet count (PC) and lymphocyte count (LC) were determined in fresh blood using Auto-Hematoanalyzer (model pocH-100i).

Immunoglobulin and progesterone levels in the serum were determined by GeNei™ Antibody Capture ELISA kit (Cat. No. 106199) and Pathozyme™ ELISA-based Progesterone Hormone detection kit and expressed in mg/ml and ng/ml serum sample respectively.

The pelleted macrophages cells that were isolated as previously mentioned, were counted after appropriate dilution through Neubauer's chambers as follows

No. of cells/ml = Average no. of cells at four corners of Neubauer's chamber × dilution factor × 10^4.

The cell concentration was adjusted to 2×10^7 cells/mm^3 by adding PBS and expressed in million cells/ml. Macrophage viability was observed using Trypan blue dye[15], an ionizable stain. Briefly 1 ml of 0.1% of the dye was added to an equal volume of cell suspension. The number of unstained (viable) and stained (non viable) cells was counted in a Neubauer's chamber using phase contrast microscope (Motic) and the percentage of viable cells was calculated using the following formula

$$\text{Percentage of viable cells} = \frac{\text{Number of viable cells}}{\text{Number of total cells}} \times 100.$$

Measure of phagocytic activity of a cell, i.e. phagocytic index was also determined[15]. In brief, *Escherichia coli* culture was centrifuged at 1500 *g* for 10 min, washed twice with PBS and the number of bacterial cells was adjusted equal to the number of macrophages/ml. Bacterial suspension was then heat-killed in boiling water bath (Sonar, New Delhi) for 30 min, cooled and then stored at –20°C till further use. Next, 6.6 µl of 10% pooled serum (for opsonization) and 200 µl killed bacterial suspension were incubated for 5, 10 and 15 min at 25°C, followed by the addition of 100 µl terminator reagent, i.e. ice-cold Hank's balanced salt solution (HBSS), and then centrifuged and the palate was resuspended in PBS. Permanent slides were prepared and the number of *E. coli* associated with macrophages was counted and the phagocytic index calculated by using the following formula

$$\text{Phagocytic index} = \text{Number of incorporated bacilli} \times 100/200 \text{ pmns.}$$

where pmns is polymorphonuclear leucocytes/neutrophils.

Table 2. Effect of *Achyranthes aspera* leaf and root extracts on haematological parameters in arsenic-intoxicated mice

Groups ⇒ Parameters ⇓	1 (Control)	2 (SA treated)	2a (SA + AALE-L)	2b (SA + AALE-H)	2c (SA + AARE-L)	2d (SA + AARE-H)
TEC	7.30 ± 0.40	5.23 ± 0.15**	5.4 ± 0.37**	5.7 ± 0.26**[b]	6.3 ± 0.45**[a]	6.1 ± 0.2**[a]
TWC	3.06 ± 0.05	4.26 ± 0.05**	4.06 ± 0.11**	3.73 ± 0.20**[a]	3.4 ± 0.25**[a]	3.7 ± 0.10**[a]
PC	631 ± 16.46	754 ± 4.61**	748 ± 0.00**	751 ± 4.16**	724 ± 5.13**[a]	729 ± 1.0**[a]
Hb	11.8 ± 0.05	10.2 ± 0.11**	11.2 ± 0.23**[a]	11.5 ± 0.10**[a]	11.5 ± 0.05**[a]	11.4 ± 0.05**[a]
HCT	38.0 ± 0.18	27.3 ± 0.80**	28.55 ± 2.04**	29.76 ± 1.44**[b]	33.07 ± 2.41**[a]	31.85 ± 1.02**[a]
MCV	54.7 ± 0.06	50.7 ± 0.13**	50.38 ± 0.36**	50.23 ± 0.83**	51.51 ± 1.20**	50.7 ± 0.79**
MCH	17.8 ± 0.12	14.9 ± 0.24**	14.83 ± 0.09**	15.2 ± 0.43**	16 ± 0.91**[b]	14.73 ± 0.47**
MCHC	32.2 ± 0.17	31.0 ± 0.1**	30.4 ± 0.40**[a]	30.83 ± 0.30**	31.83 ± 0.11*[a]	31.23 ± 0.05**
Lymphocyte count	1.65 ± 0.09	0.86 ± 0.04**	1.01 ± 0.03**[a]	1.15 ± 0.03**[a]	1.18 ± 0.026**[a]	1.32 ± 0.08**[a]

All values are mean ± S.E. ($n = 8$), *$P < 0.05$, **$P < 0.001$ vs control group; [a]$P < 0.001$, [b]$P < 0.05$ vs sodium arsenate-treated group.
TEC, Total erythrocyte count ($\times 10^6$ cells/mm^3); TWC, Total white blood corpuscles ($\times 10^3$ cells/mm^3); PC, Platelet count ($\times 10^3$ cells/mm^3); Hb, Haemoglobin (g/dl); HCT, Haematocrit value (%); MCV, Mean cell volume (fL); MCH, Mean cell haemoglobin (pg); MCHC, Mean cell haemoglobin concentration (g/dl); Lymphocyte ($\times 10^3$ cells/mm^3).

All results were expressed as mean ± S.E. The data were analysed for multiple comparisons between different experimental groups using one-way ANOVA; Post Hoc; Tukey's test of Statistical Package for Social Science Program (SPSS.16). $P < 0.05$/$P < 0.001$ was considered as significant.

As we can be seen from Table 2, haematological parameters show a significant ($P < 0.001$) decrease in TEC, Hb, HCT, MCH, MCHC and LC, while TWC and PC show significant ($P < 0.001$) increase in SA-treated mice compared with healthy mice.

Aqueous *A. aspera* leaf extract (AALE) at both doses, i.e. 100 and 200 mg/kg body wt (groups 2a and 2b) shows significant ($P < 0.05$; only in case of high dose) increase in TEC, HCT, Hb and lymphocyte count ($P < 0.001$) compared to SA-treated group. At low dose, there is a significant ($P < 0.001$) decrease in MCHC level, whereas at high dose, significant ($P < 0.001$) decrease is observed in WBCs count against SA-intoxicated mice. PC decreased insignificantly at both doses of leaf extract, but MCH shows insignificant decrease at only low dose. In contrast, slight insignificant increment at high dose of AALE is observed against SA-treated mice. MCV value also shows insignificant decrease at both doses of AALE compared with SA-treated group.

Aqueous *A. aspera* root extract (AARE) at low dose; 100 (group 2c) and 200 mg/kg body wt (group 2d) shows significant ($P < 0.001$) increase in TEC, Hb, HCT and LC, whereas MCV increases insignificantly at low dose, but decreases at high dose compared with SA-treated group in which it significantly ($P < 0.001$) decreases in comparison to control. TWC and PC significantly ($P < 0.001$) decrease when compared to group 2. Similarly, low dose of AARE shows significant ($P < 0.001$) increment in MCH and MCHC respectively, compared with SA-intoxicated mice.

Figures 1–5 indicate that various immunological parameters show a significant ($P < 0.001$) decrease in progesterone level, macrophage yield, viability and phagocytic index, but insignificant reduction in immunoglobin (Ig) level in SA-intoxicated mice in comparison to control group. AALE at both doses shows significant ($P < 0.001$; $P < 0.05$) reduction in progesterone level, macrophage yield and viability along with phagocytic index in comparison to SA-treated group (group 2). In comparison, total Ig level is found to significantly ($P < 0.001$) increase at low dose, but no effect is seen at high dose.

AARE extract at both doses shows significant ($P < 0.05$; $P < 0.001$) reduction in macrophage yield, viability and Ig level (Ig level at only at low dose) in comparison to SA-intoxicated group. AARE at high dose causes significant ($P < 0.05$; $P < 0.001$) reduction in phagocytic index and progesterone level against SA. Low dose of AARE is not as effective as high dose. So, all the results clearly depict the modulating efficacy of *A. aspera* in contrast to immunological parameters.

Nowadays, living beings have direct exposure to different risk factors like heavy metal accumulation, pathogenic agents and various mycotoxins which impair immune function. This problem can be controlled by the use of various therapeutic agents or immune modulating agents.

It is now well documented that medicinal plants provide an alternate and safe treatment against various health problems[16]. Herbal drugs are known to have immune modulating activity. These therapeutic agents of plant origin act by stimulating or suppressing both specific and non-specific immunity[17,18]. Alteration in various haematological indices provides early indication of arsenic toxicity in mice. Reduction in TEC, Hb level, haematocrit value, etc. in arsenic-intoxicated mice may be associated with anaemia[19,20], that further may be attributed to depleted RBCs survival because of impairment in various processes like reduced Hb production in bone marrow, decreasing delta-aminolevulinic acid dehydratase activity[21], reduced RBCs formation and increased membrane fragility[22]. The results of the present study also show that decrease in lymphocyte count may be associated with lymphopenia, i.e. decrease in mean cellular lifespan and impaired proliferative capacity of lymphocytes[23].

Increase in TWC might be due to over-expression of myeloid lineage cells that impart phagocytosis, inflammation, etc. Increase platelet count may lead to thrombocytosis, which is due to failure in regulation pathway of platelet production[24]. *A. aspera* extracts have the potential to restore PC level. This indicates their ability to deal with thrombocytosis. Hence, we can conclude that leaf and root extracts of *A. aspera* have the capacity to restore the level of various haematological indices.

Most of the health problems are associated with immunological imbalances in both cell-mediated and humoral-mediated immune systems[25]. Immune modulation is the process in which immune modulating agents can either enhance or suppress the immune response of an organism by interfering with the functions of cells. If immune reaction becomes enhanced after the administration of a drug, then it is known as an immune-stimulating drug and the

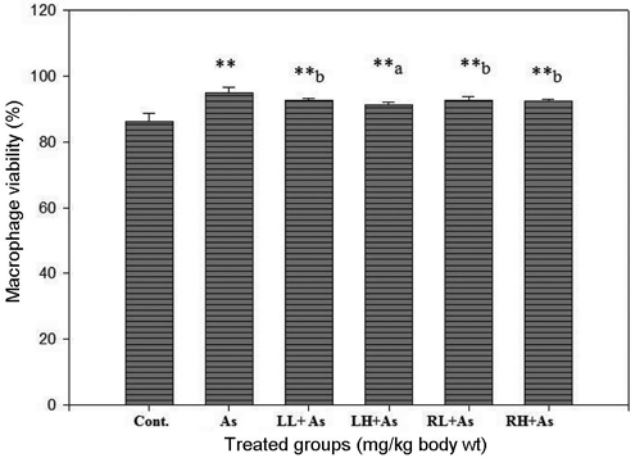

Figure 3. Effect of *A. aspera* extracts on percentage of macrophage viability in arsenic-intoxicated mice. $^{**}P < 0.001$ versus control group; $^{a}P < 0.001$, $^{b}P < 0.05$ versus sodium arsenate-treated group.

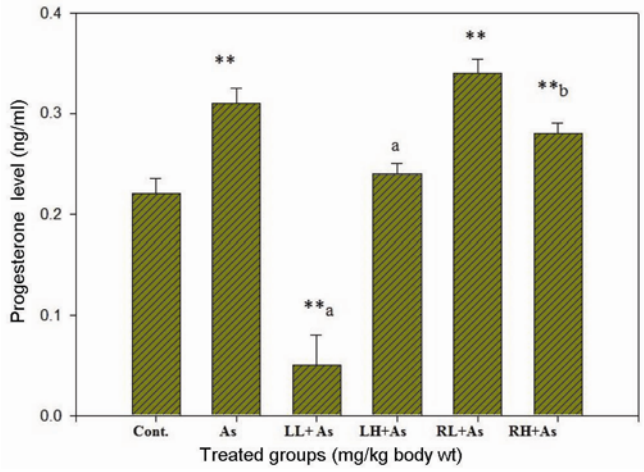

Figure 1. Effect of *Achyranthes aspera* extracts on progesterone level (ng/ml) in arsenic-intoxicated mice. Cont, Control; As, Sodium arsenate-treated; LL, Leaves low dose; LH, Leaves high dose; RL, Roots low dose; RH, Roots high dose. $^{**}P < 0.001$ versus control group; $^{a}P < 0.001$, $^{b}P < 0.05$ versus sodium arsenate-treated group.

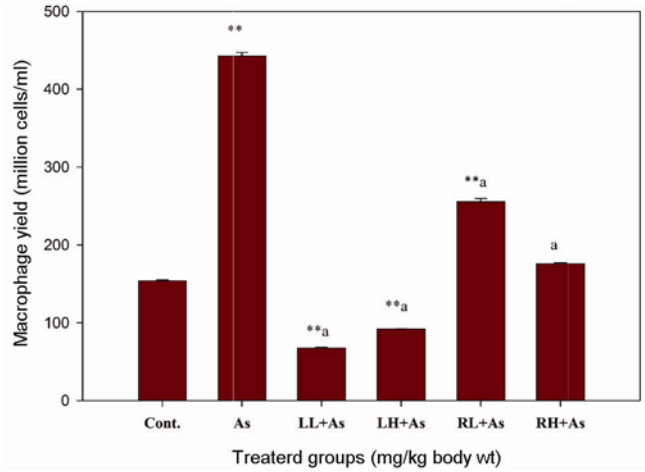

Figure 4. Effect of *A. aspera* extracts on macrophage yield (million cells/ml) in arsenic-intoxicated mice. $^{**}P < 0.001$ versus control group; $^{a}P < 0.001$ versus sodium arsenate-treated group.

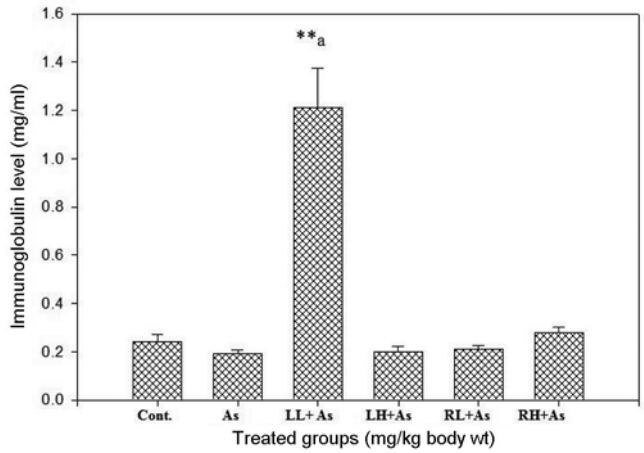

Figure 2. Effect of *A. aspera* extracts on immunoglobulin level (mg/ml) in arsenic-intoxicated mice. $^{**}P < 0.001$ versus control group; $^{a}P < 0.001$ versus sodium arsenate-treated group.

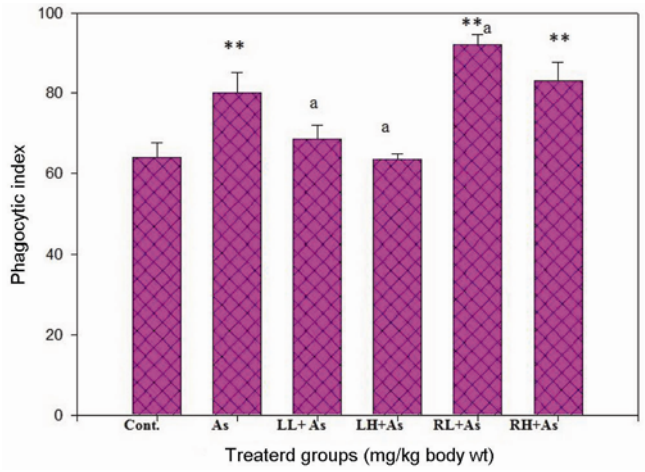

Figure 5. Effect of *A. aspera* extracts on phagocytic index in arsenic-intoxicated mice. $^{**}P < 0.001$ versus control group; $^{a}P < 0.001$ versus sodium arsenate-treated group.

process is known as immune-stimulation. An immune-stimulative drug stimulates various specific and non-specific immune system[26,27]. Sometimes in response to various factors, the resistance power against various pathogens is reduced which is known as immune-suppression[28]. In some cases, *A. aspera* aqueous leaf and root extracts showed immune modulating activity by normalizing the macrophage viability, yield and phagocytic index when compared to SA-intoxicated mice to exert their protective role. From this study, it is also evident that both extracts of *A. aspera* increased Ig level against arsenic intoxication. These findings suggest the possible protective role of *A. aspera* as a first line of defence that activates the lymphoid system.

AARE shows more normalized level of phagocytic index in comparison to leaf extract against SA toxicity. Phagocytic index is a measure of phagocytic activity of macrophages and granulocytes. Phagocytosis is processed by secretion of various cytokines like GM-CSF and IL-1 that in turn stimulate other cells (neutrophills). So, this study reveals that *A. aspera* extracts may also provide host defence by exerting anti-inflammatory and wound-healing response[29–33]. This assumption is significantly correlated with previous studies on various extracts of *A. aspera*, including aqueous extract[34–36]. Various agents like curcumin, compounds in *Viscus album*, etc. are considered as good anti-inflammatory agents. One report suggested that *V. album* shows its anti-inflammatory response by inhibiting cytokine-mediated COX-2 expression[37]. So the crude extract of *A. aspera* might possess some active compounds that interfere with PEG-2 biosynthesis. This may lead to a decrease in the expression of COX-2.

The increment in antibody titre in mice with root and leaf extracts of *A. aspera* may be due to the stimulation of macrophages and B-lymphocytes[28,38]. The humoral immunity involves the interaction of B cells with antigen, and subsequent proliferation and differentiation into plasma cells that secrete antibodies. These antibodies either directly neutralize the antigen or form complexes with the antigen which is then ingested by phagocytic cells. Elevation in steroidal hormone level, i.e. progesterone is also observed in arsenic-intoxicated mice, but a drastic change is observed after treatment with *A. aspera* leaf and root extracts which indicates that it reaches a level approximately equal to the normal. Presence of various phytoconstituents in *A. aspera* might be responsible for immune modulating activity[39]. These results suggest that leaf and root extracts of *A. aspera* are highly effective against arsenic-induced toxicity.

The findings of the present study show that both extracts of *A. aspera* have useful biological properties as indicated by the significant obliterations in haematological and immunological indices. Studies are required on isolation and characterization of various phytoconstituents that could have a possible role in poly-drug formulation to enhance the biological activity.

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Collembolans and mites communities as a tool for assessing soil quality: effect of eucalyptus plantations on soil mesofauna biodiversity

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This study aimed to assess the population dynamics of collembolans and mites in an area under eucalyptus cultivation and native grassland, and their use as bioindicators to quantify changes in soil quality. Soil samples were collected monthly and the micro-arthropods were extracted by the Berlese-Tullgren modified funnel method. There were differences in the abundance of mites and collembolans in the area, showing that eucalyptus alter the diversity of the micro-arthropods edaphic. The abundance of mites and collembolans was found to be an excellent tool for studying the impact of farming on edaphic biodiversity.

Keywords: Acari, biological indicators, environmental change, springtails.

SOIL is a critical part of the terrestrial ecosystem, and supports several forms of life[1]. Monitoring soil quality is an increasingly more relevant topic within research units. Most concepts related to soil quality have been based on the premise that the various soil components are integrated and depend on each other to fulfill their specific function. Soil quality is the result of continuous conservation and degradation processes, and it represents the capacity of soil to function as a healthy living ecosystem[2]. Soil biological quality is the ability to support and shelter a wide diversity of edaphic organisms. This is essential to maintain the integrity of terrestrial ecosystems and help them combat issues such as climate change, pest infestation, pollution and agriculture[1].

The abundance and diversity of collembolans have been widely used to assess the environmental impact of pollutants in the soil[3] or land-use effects[4]. Mites and collembolans are micro-arthropods belonging to the meso-

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