Summary

Soil and the organisms that live in it provide a number of services to human society. Most of our food is grown in it and we build our houses on it. Soil pollution can severely impact these functions and can threaten human and animal health. With the large number of chemicals manufactured by today’s modern society it is important that the impact of these potential pollutants can be assessed in a fast and sensitive manner. Gene expression analysis in selected, ecological relevant test animals may be a fast and sensitive method to test the effect chemicals and pollutants may have on animal and human health. Soil however comes in many distinct forms that have very different characteristics. These characteristics are important factors that may cause effects on gene expression or may influence pollutant properties. The main objective addressed in this thesis is how soil properties influence gene expression in the springtail *Folsomia candida* and how they affect the toxicity of soil pollutants. These results can be used to evaluate the effectiveness of a soil quality based on gene expression in *F. candida*.

In chapter two a set of reference genes is described that may be used for gene expression studies in the collembolans *Folsomia candida* and *Orchesella cincta*. These genes are especially important in quantitative real-time polymerase chain reaction (Q-PCR) experiments where expression differences of a gene of interest between different treatments are measured by comparing them to a gene that is unaffected by the treatment. Some genes are thought to have a stable expression pattern regardless of outside influences such as heat or cold treatment or chemical exposures. These genes, also called housekeeping genes, are highly sought after as reference genes for Q-PCR experiments. A set of potential reference genes was developed for both collembolan species and gene expression stability was investigated by exposing collembolans to a set of treatments both abiotic (heat, drought, soil pH) and chemical (cadmium and phenanthrene) and measuring gene expression with Q-PCR. When gene expression stability was ranked it became apparent in both collembolan species that there were differences in the top three most stable genes between the different treatments. The conclusion from this data is that universally stable reference genes are very hard to find, if they even exist at all. When performing Q-PCR experiments on genes of interest it is therefore necessary to investigate which reference genes are the most stable to be with the used with the selected treatments.
Chapter 3 deals with abiotic stress effects on *Folsomia candida* transcriptional regulation. Chemicals in the soil are influenced by soil abiotic factors. Soil pH for example, influences the uptake availability of heavy metals. In metal containing soils with a low pH, metal ions are free to be taken up by animals and plants and therefore the toxic effect of these metals can be greater in low pH soils. Soil pH in itself can also cause effects on a transcriptional level. To test the effect of soil pH on *F. candida* transcriptional regulation we exposed collembolans to four different soil pH values (3.5, 4.5, 5.5 and 6.5), all in standard OECD soil and measured the expression levels of nine, stress implicated genes. Environmental temperature can also influence gene expression because it can alter the speed at which cellular processes take place. Higher temperature may also impair protein folding which causes stress. Collembolans were therefore also exposed to four different temperatures (0, 10, 20 and 30 ºC) and the same panel of genes was tested. In the pH experiment only one gene responded to differences in soil pH. This gene is a vacuolar ATPase which transports protons across the cell membrane. In the temperature experiments only Heat Shock Proteins (HSP) 40 and 70 were affected. Remarkably, they were not only up-regulated at 30 ºC but also at 0 ºC. The up-regulation of HSP40 and HSP70 are novel findings and merit further investigation.

In Chapter 4 the concept of a Natural Operating Range is introduced. If gene expression levels are to be measured as valid endpoints in soil ecotoxicological testing, a reference database with information on how the test animal responds to natural, unpolluted soils has to be established. 26 Dutch field soils, all part of the biological soil indicator network (BoBI), an RIVM program, were sampled. *F. candida* was exposed to these soils and gene expression was measured with microarray analysis. A survival and reproduction test of 28 days was also performed to compare gene expression results to these original ISO-test endpoints. The differences between sandy and clay soils caused the largest effect on gene expression. Almost 20% of the genes were differentially expressed between these two soil types. Gene expression on animals exposed to the different land-uses only showed a minor effect; 12 genes were differentially expressed. In an multivariate analysis where gene expression was linked to the results from the soil chemical analysis it appeared that soil fertility was correlated with gene expression. No effect of soil-type or land-use was detected on survival of reproduction but the soil arsenic content was negatively correlated with reproduction.

In Chapter 5 the impact of an aged copper polluted field soil on *F candida* gene expression was determined. In 1980 a field in Bennekom, the Netherlands was spiked with
four copper concentrations and four pH treatments. The pH treatments were repeated over the years but there was no additional copper added to the soil. This design made this site an ideal, controlled, aged metal-polluted soil to test. All copper/pH combinations were sampled and gene expression, reproduction and growth were all measured. A minor copper induced effect was found on gene expression. 68 Genes were differentially regulated. Gene Ontology (GO) enrichment analysis showed that GO terms involved in vesicle mediated excretion were up-regulated in the high copper concentrations. The pH treatment induced a larger effect on gene expression in which 221 genes were differentially regulated. Neither copper treatment nor pH treatment had a significant effect on survival, reproduction or growth in these springtails. The results indicate that the copper concentration and availability in these soils were too low to produce a toxic effect. The gene expression test however, was able to differentiate between the pH and copper effects and was sensitive enough to detect non toxic effects.

In this thesis the effects of soil properties on Folsomia candida transcriptional regulation were discussed. Knowledge of these effects is necessary in order to separate them from effects induced by soil pollutants and chemicals. One of the remarkable finds was the large difference in genomic response to clay and sandy soils. Abiotic soils factors such as pH do exert an effect, albeit small, on gene expression. Therefore, for proper soil testing it is necessary to use a control soil that is similar to the soil of interest. This makes standard lab soils such as LUFA2.2 or OECD soil less applicable as controls in the testing of field soils when tests with many endpoints, such as gene expression analysis, are used. In this thesis a start was made to develop a NOR for the springtail Folsomia candida. To develop a complete NOR many natural soils should be tested.