The use of Higher Plants as Bio-Indicators of Environmental Pollution – A New Approach for Toxicity Screening in Albania

Anila Mesi Dizdari
Ditika Kopliku
Suzana Golemi

University of Shkodra "Luigj Gurakuqi", Faculty of Natural Sciences,
Department of Biology and Chemistry, Shkodër, Albania

Abstract *The purpose of this paper was to give an information review of some higher plants as: Allium cepa, Tradescantia genus, Vicia faba, Pisum sativum, Zea mays, Nicotiana tabacum, Lycopersicon esculentum, Lemna minor, etc., which can be properly used as bio-indicators for assessing and predicting pollution toxicity and environmental changes in Albania. Plants, apart their structure and metabolic priorities, can give essential information and data about the potential toxicity of substances, even when exposed in short term and low concentrations. They offer advantages against animals as bioindicators, because of the low cultivation cost, easier maintenance, ethically and esthetically acceptable handle. The plant assays can: be carried out under a wide range of environmental conditions, give access to the cito/genotoxic potential of known/unknown simple substances or even complex mixtures (present in water, soil and air) and have shown correlations with cytogenetic assays in mammals. In many sensitive species chemicals induce specific morphological and phyiological changes. Sometimes the same plant species may act as both indicator and accumulator for a special pollutant. Plants are direct recipients of agro-toxics and therefore important material for environmental monitoring of places affected by such pollutants. All above mentioned higher plants are part of Albanian wild and cultivated vegetation, so as a conclusion plant toxicity screening methods can provide a new approach, potentially applicable in Albania as a developing country, where chemical pollution monitoring is really expensive. Additionally simple plant bio-tests can be included in Albanian curricula.*

Key words*: higher plants, bio-indicator, environmental pollution, toxicity screening*

1. Introduction

Natural ecosystems are going to be imbalanced by the environmental pollution all around the world. The health and quality of biota is directly affected by the increasing discharge of different kind of pollutants into the environment. Adverse ecological effects from environmental pollutants occur at all levels of biological organization. The effects can be global or local, temporary or permanent, acute or chronic. The most serious effects involve loss in production, changes in growth, development and/or behavior, altered diversity or community structure, changes in system processes (such as nutrient cycling), and losses of valuable species. These ecological losses in turn may be economically, esthetically or socially important. (Wolska et al., 2008). The pollutants cause toxic impact on the biocenosis having toxins directly by the biotope or eating food which has accumulated toxins, and as a consequence mutagenic developing changes at cellular level.
Different tests have been designed for evaluating the potential of adverse ecological impact. Understanding how these tests can be used to prevent environmental problems caused by pollutants is the basis for ecological risk assessment research.

Genotoxic and mutagenic effects have shown to be the most worrying, due to pollutants capacity to induce genetic damage, which can lead to several health problems and also affect future generations, because these alterations can be inheritable (Ribeiro, 2006). That is the reason why the necessity to identify compounds that react with DNA in order to assure the environmental quality has led to the development of several genotoxicity and mutagenicity assays in a wide range of organisms.

The use of living organisms (fish, algae, etc.) to screen pollution degree mainly in water environment, started in the middle of last century. At late 1970s, firstly in the USA and after that in Western Europe, standard rapid ecotoxicological tests developed significally according to ISO, OECD standards (Girling et al., 2000; Blaise & Ferard, 2005; Wadhia et al., 2007). Toxicological monitoring of ecosystems totally fullfil chemical monitoring and gives preleminary allert in cases of danger, limiting or eliminating negative influences on the biota (UN/ECE, 2000; OECD, 2000; ISO/CD, 2001; Wolska et al., 2007; EPA, 2007; ISO, 2012).

Plants can successfully be used as bioassays of the level and type of air, soil and water pollution (Nilan, 1978; Cole & Smith, 1984; Kristen, 1997). There are a lot of advantages of the plant test systems which relate to: reproductive nature, possibility to be applied *in vivo, in vitro* and *in situ*; method standardization in controlled laboratory conditions, not requiring high volume samples, previous extraction or isolation procedure, ethically appropriate compared to animal tests and low cost, especially for developing countries as Albania. In addition to these advantages, the major plant test systems exhibit numerous genetic and chromosome changes for determining the effects of mutagens. This feature is due to the possibility of assessing several genetic endpoints, which range from point mutations to chromosome aberrations (CA) in cells of different organs and tissues, such as leaves, roots and pollen (Grant, 1994). Some of these mutations have not yet been detected in other non mammalian and mammalian test systems, but probably occur in the human organism.

The purpose of this paper was to give an information review of some higher plants as: *Allium cepa* L., *Tradescantia* genus, *Vicia faba* L., *Pisum sativum* L., *Zea mays* L., *Nicotiana tabacum* L., *Lycopersicon esculentum* Mill., *Lemna minor* L., etc., which can be properly used as bio-indicators for assessing and predicting pollution toxicity and environmental changes in Albania.

2. Review of some plant bio tests

2.1 Allium test

The use of *Allium cepa* L. (2n=16) as a test system was introduced by Levan showing disturbances in the mitotic spindle due to the use of colchicines, and several chromosome aberration types in meristematic root cells induced by different solutions of organic salts (Levan, 1938, 1945).

Allium test has many advantages, compared to other organisms: the plant material is easy to be provided, to store and to handle; the method is standardized in environmental monitoring and toxicity screening for drinking, natural (river and lake) waters, waste water, etc.; the possibility to expose the test organism directly to complex mixtures without previous treatment of the test sample; the presence of important enzymes, necessary for the activation of some promutagen; the combination of two test targets, as: toxicity by growth inhibition and mutagenity by chromosome aberrations types and frequency; the low cost of experimentally design; the compatibility with a test battery composed of prokaryotes and/or other eukaryotes, etc., (Waters & Auletta, 1981; Fiskesjö, 1985; Smaka-Kincl et al. 1996; Radić et al., 2010). Protocols have been given for using root tips from either bulbs or seeds of *Allium cepa* (Grant, 1982a).

The test has been modified, becoming technically more appropriate for the assessment of chemicals. The first adaptations of the *A. cepa* test were made by Fiskesjö (1985), making it applicable for environmental monitoring: evaluation of known and unknown, soluble and insoluble compounds in water and the assessment of complex mixture effects. Rank and Nielsen (1993) proposed new modifications to the test, making it even more efficient to analyze complex mixtures. However, all the modifications proposed by the authors were related to the evaluation of CA, which detects potentially genotoxic agents. Ma et al. (1978) proposed *Allium* test for micronuclei (MN) in root cells exposed to environmental pollutants. The analysis of CA in *A. cepa* has shown to be more efficient for investigating the action mechanisms of pollutants on DNA. (Leme et al., 2008). The International Programme on Plant Bioassays (IPPB) has acknowledged, standardized and validated *Allium* test for monitoring and testing of environment polluters.

The *A. cepa* test enables the assessment of different cytological and genetic categories, as: Mitotic Index, Phase Index, chromosome aberrations, nuclear abnormalities and micronucleus.

The decrease of MI can be considered capable to estimate the presence of cytotoxic agents in the environment and the respective pollution levels (Smaka-Kincl et al., 1996). MIs higher than the negative control (usually filtered drinking water) are results of an increase in cell division, which can be harmful to the cells, leading to a disordered cell proliferation and even to the formation of tumor tissues.(Hoshina et al., 2002).

Rank and Nielsen (1993) proposed the analysis of abnormalities only in anaphase and telophase. test in order to facilitate CA analysis for scientists who do not work in the cytology area. Anyway, the analysis of the different CA types, in all phases of the cell cycle permits a better investigation of clastogenic (chromosome bridges and breaks) and aneugenic (chromosome losses, delays, adherence, multipolarity and c-metaphases) effects on the DNA (Bolle et al., 2004).

Nuclear abnormalities (NA) evaluation, recently introduced, has shown to be a sensitive analysis, to investigate more accurately the relation of pollutant effects on the DNA. The presence of lobulated nuclei and polynuclear cells can indicate a cell death process; because these abnormalities are not observed in the next generation of *A. cepa* roots (Leme et al., 2008).

Developed by Schmid, (1975), micronucleus *A. cepa* in vivo and in vitro test (MN), is considered the most simple, cheap, rapid and effective method to analyze the mutagenic effect caused by chemicals. It results from damages in the parental cells, which can, easily observed in daughter cells in a reduced size compared to the main nucleus (Ribeiro, 2006). Since *A. cepa* presents a symmetric karyotype, with large and few chromosomes (2n = 16), MN size can be an effective parameter to assess the clastogenic (resulting from chromosome break) and aneugenic (resulting from chromosome loss) effects (Ma e t al., 1995; Cotelle et al., 1999; Ferreti et al., 2007; Leme et al., 2008).

The *A. cepa* test has been employed to detect the presence and the quantification of many environmental pollutants (present in air, soil, fresh and sea waters), such as: heavy metals, different effluents by natural processes and human activities (metal mining, metallurgical, chemical, textile, tannery, tourism industries, oil refinery, transport emissions, disposal of different kind wastes without previous treatments, pesticides, herbicides, organic wastes as fertilizer, drinking water disinfectants, detergents, etc., (Fiskesjö, 1988; Liu et al., 1995; Boorman, 1999; Cabrera & Rodriguez, 1999; Cotelle et al., 1999; Dovgaliuk et al., 2001; Chandra et al., 2004; Chandra et al., 2005; Palacio et al., 2005; Srivastava et al., 2005; Ferreti et al., 2008; Yildiz & Arikan, 2008; Souza et al., 2009; Srivastava & Mishra, 2009).

Other species of *Allium* genus (*A. sativum, A. cepa* var. *proliferum* and *ascalonicum, A. carinatum* and *A. fistulosum*) have also been used but to a much lesser extent (Grant, 1982a; Glasenčnik, et al., 2004; Vetayasuporn, 2006; Meng et al., 2007; Liu et al., 2009).

2.2 Tradescantia assays

The genus *Tradescantia* belongs to the Commelinaceae family and comprises about 500 species. A wealth of basic genetic and developmental information available on *Tradescantia* plants provides a solid framework in support of their use as biomonitors in environmental genotoxicity assays (Ma and Grant, 1982). The most frequently used clone for genotoxicity studies is #4430, which is a hybrid between *Tradescantia hirsutiflora* and *Tradescantia subacaulis*. Two assays, which are considered ideal for *in situ* monitoring and testing of airborne and aqueous mutagenic agents, are the *Tradescantia* stamen hair assay for mutations and the *Tradescantia* micronucleus assay for chromosome aberrations. Both assays can be used for *in vivo* and *in vitro* testing (Ma et al., 1985; Grant, 1994). Additionally, the *Tradescantia* assays have proven to be suitable for studies on synergism between chemicals and between chemicals and other genotoxic agents, such as radiation - a valuable property for the assessment of genotoxic risks in complex environmental situations (Shima and Ichikawa, 1995).

The *Tradescantia* micronucleus (Trad-MCN) test is based on the formation of micronuclei, resulting from chromosome breakage in the meiotic pollen mother cells of *Tradescantia* inflorescences (Mišík et al., 2010). This bioassay was originally developed as a test system for the gaseous mutagen 1,2-dibromoethane (Ma et al., 1978). Since then, it has been applied in many studies to screen the mutagenic effects of chemicals, wastewater, surface water, contaminated soil, leachates, radiation etc., mostly under laboratory conditions (Ma, 1995; Rodrigues et al., 1997; Grant, 1998; Cabrera & Rodriguez, 1999, Cotelle et al., 1999). The bioassay has also been employed for *in situ* monitoring studies of air pollutants emitted, by: waste incinerators, disposal sites (Sadowska et al., 1994; Ma et al., 1996; Fomin and Hafner, 1998) and urban traffic (Monarca et al., 1999; Guimarães et al., 2000; Isidori et al., 2003). As a consequence of its features, *Tradescantia* was proposed for indoor pollution monitoring. Several studies have assessed the sensitivity of the Trad-MCN assay for the low level of contaminants normally present in home environments, as: air fresheners, tobacco smoke, p-dichlorobenzene (moth balls) and other insecticides listed for domestic use (Harris and Ma, 1983; Ma and Harris, 1987a; Ma and Harris, 1987b; Ma et al., 1983).

The *Tradescantia* stamen-hair mutation assay (Trad-SHM) is a point mutation (mitotic) assay, in which expression of the heterozygous dominant blue character of the stamen hair cells is prevented, resulting in the appearance of the recessive pink color (Mericle and Mericle, 1971; Emmerling-Thomson and Nawrocky, 1982). In this assay, full growth of the hair was considered as equivalent to colony formation and stunted hairs. In addition to mutation (color change) being used as an endpoint, genotoxic changes such as the expression of giant, twin or triplet cells, branching of the hair and other growth anomalies were recorded along with loss of reproductive integrity as indicators of genotoxicity (Nayar and Sparrow, 1967). Pink mutation as well as loss of reproductive integrity in the stamen hairs of several species and hybrids of *Tradescantia* (Ichikawa and Sparrow, 1969) became important endpoints in the study of the genotoxic effects of soil, waste water and radiation (Nauman et al., 1976; Cabrera & Rodriguez, 1999).

The most important contribution of the Trad-SHM assay was its use on atmospheric pollution, carried out with a mobile laboratory (Schairer et al., 1979). The Trad-SHM assay evaluating the mutagenesis of chemical agents involves maleic hydrazide (Gichner et al., 1982), methyl methane sulfonate, ethyl methane-sulfonate, dimethyl sulfate (Ichikawa and Takahashi, 1978; Ichikawa et al., 1990; Sanda-Kamigawara et al., 1991), N-nitroso compounds and several organic solvents, as well as evaluations of synergistic action between chemicals and between chemicals and radiation (Gichner et al., 1988; Kuglik et al., 1994; Shima & Ichikawa, 1994; Shima & Ichikawa, 1995). In addition to studies of gaseous mutagens, the Trad-SHM assay has been used to assess the mutagenicity of aquatic environments (Lower et al., 1984; Tano, 1989).

2.3 Vicia faba test

Vicia faba L. is a well known plant used for citological, physiological, radiobiological studies and as bio-test for environmental toxicity screening. Some of its advantages are: the plant physiological properties and reactions toward external pollutant agents are well known; the material is available during the whole year, not expensive, easy to grow and to handle; the method does not require sterile conditions and expensive material or equipments, the root meristem contains a high proportion of cell in mitosis; the chromosome number is low (2n=12) and chromosomes are large enough for accurate scoring and studying of chemical substances effects on chromosome aberrations and chromatide sister exchanges frequency (Kihlman, 1975; Poschenrieder et al., 1989; Gómez-Arroyo et al., 1994).

The root tip micronucleus, chromosome aberrations and sister chromatide exchange tests, as genotoxicity assays of this plant, are the most employed on various types of contaminated materials.

V. faba micronuclei test has been firstly standardized by AFNOR, the French member organization of ISO. It can be successfully performed for both liquid phase: exposure of plants to different liquid matrix, including soil water extracts, and solid phase: direct exposure of plants to the soil, showing that the last one is more practical (Foltête, 2011).

The *V. faba* MCN test is employed to detect the presence and the quantal effects of many environmental pollutants (present mostly in soil and extracted soil water, drinking and irrigation water sources), such as: heavy metals (Cd, Cr, Cu) different effluents by natural processes and human activities: pesticides, herbicides, lanthanides- clastogenicity inducers, municipal landfill leachates, laundry detergents (Badr, 1983; de Kergommeaux et al., 1983; De Marco et al., 1986; De Marco et al., 1988; Jha & Singh, 1994; Ma e t al., 1995; Cotelle et al., 1999; Sang & Li, 2004).

The protocol standardization of Vicia faba chromosomal aberrations assay has been done in collaborative laboratory studies all around the world, concluding that it is an efficient and reliable short-term bioassay for the rapid screening of chemicals for clastogenicity (Kaul, 1969; Miadoková, 1992; Kanaya et al., 1994; Souguir, 2008; Adam & El-Ashry, 2010).
Vicia faba SCE test has been employed and is widely advised to detect mutagens and carcinogens, as: Arsenic and Cadmium salts, food preservatives, pesticides and essences (Tempelaar, et al., 1982; Gómez-Arroyo et al., 1989; Gómez-Arroyo et al., 1989; Xing & Zhang, 1990; Zhang et al., 1991; Zhang et al., 2009).

2.4 Lemna minor L. test

Water pollution by toxic micropollutants, which is predominantly the consequence of human activities (industry, agriculture and urbanization), is one of the most critical problems concerning drinking water resources and environmental protection of water bodies. That is the reason why several studies for aquatic ecosystems demonstrated that many species of duckweed, a group of free-floating freshwater plants of the family *Lemnaceae*, are able to absorb and accumulate high amount of heavy metals (especially Copper, Chromium, Nickel and Cadmium) in their biomass, producing an internal concentration several fold greater than the nutrient medium (Wang, 1987; Jain et al., 1989; Zayed et al., 1998; Miretzky et al., 2004; Khellaf & Zerdaoui, 2009).

Duckweed plants are especially suitable for use in complex effluent bioassays, and for testing herbicide pollution in the aquatic environment, lake and river pollution, sediment toxicity (Wang, 1990). Among the tools used to study effects of toxic elements on plants, growth and photosynthesis, plants as *Lemna minor* are often proposed as simple, rapid and sensitive methods, witch for some industrial effluents, results really sensitive indicator (Taraldsen & Norberg-King, 1990; AFNOR, 1996).

Common duckweed is potentially useful as an indicator of pollution, because it is a small monocot plant, grows rapidly, is easy to culture, sensitive to a wide variety of toxicants and has the ability to integrate and rapidly monitor the pollutant's variations in the water. Moreover, this plant as biotest tolerates unstable environmental conditions and exhibits high sensitivity to heavy metal toxicity. *Lemna* bioassay can be efficiently used to assess combined effects of multi metal samples, which can help explain many different interactions of metals on plant growth and metabolism. On the other hand differences in duckweed test methodology occur with regard to test types, test vessels, control tests, nutrient media, end points, and applications (U.S. EPA, 1985; Mackenzie et al. 2003). Evaluation of classical toxicity endpoints: relative growth rate, dry to fresh weight ratio and guaiacol peroxidase activity (as early indicator of oxidative stress) can be done (Horvat et al., 2007).

2.5 Pisum sativum assay

The *Pisum sativum* L. (2n=14) bioassay has been shown to be a very good plant bioassay for assessing antimitotic effects, micronuclei and chromosome aberrations both in mitosis and meiosis for somatic mutations induced by chemicals (heavy metals, pesticides, etc.), radiations, and *in situ* environmental pollutants (Jain & Sarbhoy, 1987; Grant & Owens, 2001; Souguir et al., 2008).
Current interest in the field of investigating the impact of air pollutants on agricultural crops is now centred on short-term low-level effects of the main phytotoxic gases O_3, SO_2 and NO_x on crop production. *P. sativum* chronic exposure to air pollutants can cause yield losses, changes in plant development and crop quality, reduced net growth (Ali, 2004).
P. sativum assay has also been used to evaluate photosynthesis and growth response under heavy metal stress (Hattab et al., 2009). *Pisum fulvum* (2n=14) has also been applied in clastogenic studies, but to a much lesser extent (Grant & Owens, 2001).

2.6 Nicotiana tabacum assays

Nicotiana tabacum is a widely used sensitive bio-indicator for ambient ozone (Sant'Anna, et al., 2008). Among the most useful air monitoring tools are several cultivars of *Nicotiana tabacum* L., as: *Bel-B* ozone resistant, *Bel-C* ozone-sensitive, *Bel-W3* ozone supersensitive, *cv. ZZ100,* cv. *Dynes* and cv. *Weather fleck,* which were identified as indicators for ozone and peroxyacyl nitrate (PAN), another phytotoxic oxidant (Ribas et al., 1998; Azadi & Doley 2004). Tobacco, used extensively in studying the photochemical oxidant complex (Heggestad & Darley, 1968), has proven to be an excellent monitor for several reasons. It produces new leaves continuously during the growing season. Leaves of different maturity differ in sensitivity and leaves are uniformly sensitive at a given stage of growth. New injury is easily separated visually from old injury. A *Bel-W3* plant shows characteristic, easily identifiable, and quite specific symptoms of oxidant injury. The so-called 'flecking' is made up of numerous small lesions, primarily on the upper leaf surface of fully expanded leaves. *Bel-W3* tobacco leaves injury by ozone demonstrate also the influence of soil type, soil moisture stress, light, temperature, and nutrient level of the soil (Feder, 1978).

Nicotiana tabacum plants heterozygous for the Sulfur nuclear gene (*Su*) are also used as soil pollution bio-monitors, because they can carry on photosynthesis, although not to the full extent. *Su/+* plants can be used to analyze the potential genotoxicity of ionizing radiation and toxic chemicals. The advantages are: non transgenic plants and visual analysis of growth dynamic changes correlated with increasing of dark green spots on light-green leaves (caused by various mutations, serving as an indicator of mutagenicity) can be done (Jastrebova et al., 2010).

2.7 Lycopersicon esculentum test

Tomato (*Lycopersicon esculentum* Mill.) is considered as one of the most widely grown vegetable crop in the world. Despite pathogens, one of the most constraints of tomato cultivation is the environmental pollution. Several studies have shown that *L. esculentum* (2n=24) assay is a very good plant bioassay for assessing dry weight, seed germination, radicula length, total soluble protein content, morphological features and fertility of pollens, chromosome damage both in mitosis and meiosis, somatic and gene mutations, induced by: chemicals as: pesticides, fungicides, heavy metals (Pb, Zn, Cd, Hg, etc.), ozone, boron, fluoranthene, municipal waste water sludge and different types of radiations, separately or in combined treatments. Many of these pollutant agents causes clastogenic effects in *L. esculentum.* (Tort et al., 2005; Lopez-Millan et al., 2009; Öztürk Çali, 2009; Hossain et al., 2010; Oguntimehin et al., 2010; Opeolu et al., 2010; Salam et al., 2010; Usha et al., 2010; Abida & Begum, 2011; Sumer Aras et al., 2011).
Tests using *L. esculentum* can be made for a spectrum of mutant phenotypes of which many are identifiable in young seedlings. The *Lycopersicon* bioassay has been shown to be as sensitive as other plant genotoxicity assays (Grant & Owens, 2002).

2.8 Zea mays test

Agronomic plant *Zea mays* L. (2n=20) is a very good plant bioassay for assessing physiological and biochemical responses, chromosome damage (both in mitosis and meiosis), somatic mutations and oxidative stress, induced by heavy metals and metalloids, landfill leachate and wastewater, airborne particulates, radiations, etc. Physiological and genotoxical parameters, as: fresh biomass, chlorophyll content, root colour, increased root thickness with stiffening and reduced root length, lipid peroxidation, protein oxidation and activities of antioxidant enzymes, the mitotic index,

chromosome aberrations, micronuclei and sister-chromatid exchange were evaluated (Doncheva et al., 1996; Jiang et al., 2001; Poma et al., 2002; Sang et al., 2010; Duquesnoy et al., 2010; Han et al., 2011). Maize pollen has also been employed as a monitor for environmental pollutants causing carcinogenesis (Lower et al., 1978; Plewa, 1978).
This plant has been used to study mutagenesis under both laboratory and *in situ* conditions. Maize is sensitive to a wide range of mutagens and has the capacity to activate promutagens.
Tests using *Zea mays* can be made for a spectrum of mutant phenotypes of which many are identifiable in young seedlings (Plewa, 1982; Grant & Owens, 2006).
The maize bioassay has been shown to be as sensitive and as specific assay as other plant genotoxicity assays, like: *Hordeum vulgare*, *Vicia faba, Crepis capillaris, Pisum sativum, Lycopersicon esculentum* and *Allium cepa* and should be considered in further studies in assessing clastogenicity (Liu et al., 2005; Grant & Owens, 2006).

3. Environmental pollution and plant bio-monitoring - a new approach for toxicity screening in Albania

Before 1990' environmental situation in Albania did not fulfill any protection standards and the result of industry was air pollution, water pollution, soil contamination, extermination of the flora and fauna and direct impact on the health of the populations. Later one tons of poisonous substances, abandoned in derelicts factories, discharged in the environment. Moreover increase in air, water, and soil pollution was caused by second-hand cars, trash burning, waste production by consumption increase and individuals and businesses irresponsibility, which illegally dumped waste on fields, forests, rivers, sea and waterways, giving rise to harmful and dangerous bacteria and algae and producing a lot of methane, which is deadly for water organisms, and a greenhouse gas many times more powerful than CO_2 (UNEP, 2000; AFP, 2004).

Major sources of air pollution in Albania include: increasing traffic (using mainly leaded petrol and falling on air bubbles of NO_x, SO_2, CO and CO_2 above the tolerance level set by the World Health Organizations) combined with badly maintained roads, energy production (oil and gas extraction and refining), numerous cement- and metal-producing factories (emitting thousands of tons of dust per year), rapid urban development, construction industry (spreading particulate matter). (SSC & REC/CEE, 2000)

Contamination of soil and water is another major environmental problem in our country, mainly caused by: abandoned factories, mining enterprises, deforestation, soil erosion, changes in sediment supply, hydroelectric dams and channel modifications, uncontrolled migration and settling in lowland areas, tourism activities, illegal hunting, fishing and collection of essential oil plants, , waste dumps. Waste management is at a low level, very little recycling of waste is undertaken and the main method of disposal is dumping on land. There are no collection systems in rural areas and small towns, and no system for management of hazardous waste. The degradation water quality of many natural sources in Albania (rivers, lakes, streams) is significant. At the same time water of some rivers is used for drinking water supply and irrigation. Some of the hot spots of hazardous substance are the chemical plant in Porto Romano, the plastics plant in Vlora (mercury), the nitrate plant in Fier (arsenic), the Ballshi Oil Refinery and Sharra landfill. (NEA, 2000; SSC & REC/CEE, 2000; Floqi et al., 2007)

Negative impact of environmental pollution includes: losses in species, increasing number of endangered species, local extinction of grass meadows, respiratory and cardiovascular diseases, celebral weakness, babies born with deformities, cancer and huge mutagenic problems in the future.

Nowdays there is still present a weak implementation of environmental protection laws and lack of control over them. Only a small part of Albania's territory has protected status, which is considered too small to have a long-term impact on biodiversity protection. Access to safe water and adequate sanitation are other significant public health problems to be solved by government institutions. Standard targeted chemical analyses do not provide information about the biological effects of pollutants, determined analytically (Smaka-Kincl et al., 1996; Kungolos et al., 2006; Žegura et al., 2009). In this context, toxicity and genotoxicity tests employing microorganisms, plant cells and mammalian cells, alone or in combination with chemical analysis, are widely used all around the world, but not in Albania. Unlike physico-chemical analysis, saprobiological, cytogenetical and genotoxical analysis in plants are currently not an integral part of the environment quality monitoring programs, conducted by Ministry of Environment, Forestry and Water Administration (National Environmental Strategy, 2006 and the Environment Sector and Cross Cutting Strategy, 2007 and National Water Council).

The *Allium* test has been recently introduced in Albania to screen the chemical water quality of Shkodra Lake, Buna and Drini rivers, Malësia e Madhe and NënShkodra lowland water bodies (Kopliku et al. 2011; Mesi & Koliku, 2011; Mesi et al., 2011; Kopliku et al. 2012; Mesi et al., 2012).

All above mentioned higher plants are part of Albanian wild and cultivated vegetation, so plant toxicity screening methods can provide a new approach, potentially applicable in Albania as a developing country, where chemical pollution monitoring is really expensive. Additionally simple plant bio-tests can be included in Albanian curricula and environmental education projects (Xhuveli et al., 1987; ASHRSH, 2000; Nasto Th. & Bardhi, N., 2004; Dhora & Rakaj, 2010; MAFCP, 2011).

4. Conclusions

- This is the first review about plant bioassays, wich can be used for environmental toxicity screening in Albania.
- Moreover, for the possibility of assessing several physiological and genetic endpoints, theses tests also enable the evaluation of presence (bioindication and bioaccumulation) and action mechanisms of the pollutants on the exposed plant organisms.This is rather crucial even for the fact that most part of above mentioned plants are important crops for albanian agriculture.
- The results of provided methods for screening environmental contamination included in this paper, can serve as a warning to other test systems.
- Researchers employed in the respective field can find valuable information easily applicable in our country's institutions responsable for environment protection.
- In addition plant bioassays described and analyzed in this review can be included into the academic curricula and concretely contribute to scientific education of new scientists generation and the ammelioration of environment situation in our deloping country.

References

Abida, B. & Begum, A. (2011). Evaluation of Municipal Sewage sludge vermicompost on two cultivars of tomato (*Lycopersicon esculentum*) plants. *International Journal of Chemical Technology Research*, 3 (3), 1184-1188.

Adam, F. I. M. & El-Ashry, Z. M. (2010). Evaluation of Genotoxicity of 4-n-Nonylphenol using *Vicia faba* L. *Journal of Biological Sciences,* 10, 368-372.

AFNOR (1996). Determination of the inhibitory effect on the growth of *Lemna minor* XP T 90-337.

AFP (2004). Albania at risk of disaster from heightened pollution levels. [Online] Available: http://www.terradaily.com/2004/040525022741.3zfr2azt.html

ASHRSH (2000). Flora e Shqipërisë (IV, pp. 394-395). *Akademia e Shkencave e Republikës së Shqipërisë, Instituti i Kërkimeve Biologjike*, Tiranë, Albania.

Ali, A. A. (2004). An open-top chamber study to evaluate the impact of airborne pollutants on the growth of pea (*Pisum sativum* L.) cultivars at three sites of Sharkia Province, Egypt. *Egyptian Journal of Biology,* 6, 52-61.

Azadi, M. & Doley, D. (2004). Biological indicators of air quality in Brisbane, Australia. *International Journal of Environmental Science & Technology,* 1(1), 59- 68.

Badr, A. (1983). Cytogenetic activities of a triazine herbicide in root tips of *Allium cepa* and *Vicia faba*. *Mutation Research/Genetic Toxicology*, 117(1-3), 173-182.

Blaise, C. & Ferard, J. F. (2005). *Small-scale fresh water toxicity investigations* (2nd ed.). Springer.

Bolle, P., Mastrangelo, S., Tucci, P. & Evandri M. G. (2004). Clastogenicity of atrazine assessed with the *Allium cepa* test. *Environmental & Molecular Mutagenesis*, 43, 137-141.

Boorman, G. A. (1999). Drinking water disinfection by-products: review & approach to toxicity evaluation. *Environmental Health Perspectives*, 107, 207-217.

Cabrera, G. L. &. Rodriguez, D. M. G (1999). Genotoxicity of soil from farm and irrigated with wastewater using three plant bioassays. *Mutation Research*, 426, 211-214.

Chandra, S., Chauhan, L. K. S., Pande, P. N. & Gupta, S. K. (2004). Cytogenetic effects of leachates from tannery solid waste on the somatic cells of *Vicia faba. Environmental Toxicology*, 19, 129-133.

Chandra, S., Chauhan, L. K. S., Murthy, R. C., Saxena, P. N., P&e P. N. & Gupta, S. K. (2005). Comparative biomonitoring of leachates from hazardous solid waste of two industries using *Allium* test. *Science of the Total Environment*, 347, 46-52.

Cole, M. & Smith, R. F. (1984). Vegetation as Indicator of Environmental Pollution. *Transactions of the Institute of British Geographers*, 9 (4), 477-493.

Cotelle, S., Masfaraud, J. F. & Ferard, J. F. (1999). Assessment of the genotoxicity of contaminated soil with the *Allium/Vicia*-micronucleus & *Tradescantia*-micronucleus assays. *Mutation Research*, 426, 167–171.

De Marco, A., Romanelli, M., Stazi, M. A. & Vitaglian, E. (1986). Induction of micronucleated cells in Vicia faba and *Allium cepa* root tips treated with nitrilotriacetic acid (NTA). *Mutation Research/Genetic Toxicology,* 171(2-3), 145-148.

De Marco, A., Paglialunga, S., Rizzoni, M., Testa, A & Trinca, S. (1988). Induction of micronuclei in *Vicia faba* root tips treated with heavy metals (cadmium and chromium) in the presence of NTA. *Mutation Research/Genetic Toxicology*, 206(3), 311-315.

Dhora, Dh. & Rakaj, M. (2010). List of plant and animal species of the Shkodra Lake (pp. 23). *Camaj-Pipa*, Shkodër, Albania

Doncheva, S., Nikolov, B. & Ogneva, V. (1996). Effect of excess copper on the morphology of the nucleus in maize root meristem cells. *Physiologia Plantarum*, 96(1), 118-122.

Dovgaliuk, A. I., Kaliniak, T. B. & Blium, B. (2001). Assessment of phyto and cyto-toxic effect of heavy metals & aluminum compounds using onion apical meristem. *Tsitologiia i genetika*, 35, 3-9.

Duquesnoy, I., Champeau, G. M., Evray, G., Ledoigt, G. & Piquet-Pissaloux, A. (2010). Enzymatic adaptations to arsenic-induced oxidative stress in *Zea mays* and genotoxic effect of arsenic in root tips of *Vicia faba* and *Zea mays. Comptes Rendus Biologies*, 333 (11), 814-824.

Emmerling-Thompson, M. & Nawrocky, M. M. (1982). Evidence of gametic mutation for newer color in *Tradescantia. Environmental* & Experimental Botany, 22, 403-408.

EPA, (2007). Solution to analytical chemistry problems with Clean Water Acts methods. U.S. EPA Report (EPA. 821-R-07-002), Washington DC, USA.

Feder, W. A. (1978). Plants as bioassay systems for monitoring atmospheric pollutants *Environmental Health Perspectives*, 27, 139-147.

Feretti, D., Zerbini, I., Zani, C., Ceretti, E., Moretti, M. & Monarca, S. (2007). *Allium cepa* chromosome aberration & micronucleus tests applied to study genotoxicity of extracts from pesticide-treated vegetables & grapes. *Food Additives & Contaminants*, 24(6), 561-72.

Feretti, D., Zerbini, I., Ceretti, E., Villarini, M., Zani, C., Moretti, M., Fatigoni, C., Orizio, G., Donato, F. & Monarca, S. (2008). Evaluation of chlorite and chlorate genotoxicity using plant bioassays and in vitro DNA damage tests. *Water Resrarch*, 42, 4075-4082.

Fiskesjö, G. (1985). The *Allium test* as a standard in environmental monitoring. *Hereditas,* 102, 99-112.

Fiskesjö, G. (1988). The *Allium* test - an alternative in environmental studies: the relative toxicity of metal ions. *Mutation Research*, 197, 243-260.

Floqi, T., Vezi, D. & Malollari, I. (2007). Identification and evaluation of water pollution from Albanian tanneries. *Desalination*, 213, 56–64.

Foltête, A-S., Dhyèvre, A., Férard, J-F. & Cotelle, S. (2011). Improvement of *Vicia*-micronucleus test for assessment of soil quality: A proposal for international standardization. *Chemosphere*, 85, 1624-1629.

Fomin, A., & Hafner, C. (1998). Evaluation of genotoxicity of emissions from municipal waste incinerators with *Tradescantia*-micronucleus bioassay (Trad-MCN). *Mutation Research*, 414, 139-148.

Gichner, T., Veleminsky, J. & Rieger, R. (1988). Antimutagenic effects of diethyl dithiocarbamate towards maleic hydrazide and N-nitrosodiethy-lamine-induced mutagenicity in the *Tradescantia* mutagenicity assay. *Plant Biology,* 30, 14-19.

Girling, A. E., Pascoe, D., Janssen, C. R., Peither, A., Wenzel, A. A., Schafer, H., Neumeier, B., Mitchell, G. C., Taylor, E. J., Maund, S. J., Lay, J. P., Juttner, A. I., Crossland, N. O., Stephenson, R. R. & Persoone, G. (2000). Development of methods for evaluating toxicity to freshwater ecosystems. *Ecotoxicology and Environmental Safety*, 45, 148.

Glasenčnik, E., Ribarič-Lasnik, C., Savinek, K., Zaluberšek, M. Mueller, M & Batič, F. (2004). Impact of air pollution on genetic material of shallot (Allium cepa L. var. ascalonicum) exposed at differently polluted sites in Slovenia. *Journal of Atmospheric Chemistry,* 49, 363-376.

Gómez-Arroyo, S., Abarca-Hernandez, J. C., Cortës-Eslava, J. & Villalobos-Pietrini, R. (1989). Sister chromatid exchanges induced by Cadmium in *Vicia faba. La Revista Internacional de Contaminación Ambiental*, 5, 71-82.

Gómez-Arroyo, S., Abarca-Hernandez, J. C., Cortés-Eslava, J. & Villalobos-Pietrini, R. (1997). Sister chromatid exchanges in *Vicia faba* induced by arsenic-contaminated drinking water from Zimapan, Hidalgo, Mexico. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 394(1-3), 1-7.

Grant, W. F. (1982a). Chromosome aberration assays in *Allium*. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutation Research*, 99(3), 273-91.

Grant, W. F. (1982b). The *Tradescantia* - adventurous plants. *Herbarist,* 48, 36-14.

Grant, W. F. (1994). The present status of higher plane bioassay for the detection of environmental mutagens. *Mutation Research/Genetic Toxicology*, 310, 175-185.

Grant, W. F. (1998). Higher plant assays for the detection of genotoxicity in air polluted environments. *Ecosystem Health*, 4, 210-229.

Grant, W. F. & Owens, E. T. (2001). Chromosome aberration assays in *Pisum* for the study of environmental mutagens. *Mutation Research*, 488 (2), 93-118.

Grant, W. F. & Owens, E. T. (2002). *Lycopersicon* assays of chemical/radiation genotoxicity for the study of environmental mutagens. *Mutation Research*, 511 (3), 207-37.

Grant, W. F. & Owens, E. T. (2006). *Zea mays* assays of chemical/radiation genotoxicity for the study of environmental mutagens. *Mutation Research/ Review,* 613 (1), 17-64.

Guimarães, E. T., Domingos, M., Alves, E. S., Caldini, Jr. N., Lobo, D. J. A., Lichtenfels, A. J. F. C. & Saldiva, P. H. N. (2000). Detection of the genotoxicity of air pollutants in and around the city of São Paulo (Brazil) with the *Tradescantia*-micronucleus (Trad-MCN) assay. *Environmental and Experimental Botany,* 44, 1-8.

Han, M., Li, G., Sang, N. & Dong, Y. (2011). Investigating the bio-toxicity of coking wastewater using *Zea mays* L. assay. *Ecotoxicological and Environmental Safety*, 74 (4), 1050-6.

Harris, M. M. & Ma, T. H. (1983). *Tradescantia* micronucleus test on the mutagenicity of airfresheners. *Environmental Mutagen,* 4, 65.

Hattab, S., Dridi, B., Chouba, L., Ben Kheder, M. & Bousetta, H. (2009). Photosynthesis and growth responses of pea *Pisum sativum* L. under heavy metals stress. *Journal of Environmental Sciences*, 21, 1552-1556.

Heggestad, H. E. & Darley, E. F. (1968). Plants as indicators of the air pollutants ozone and PAN. Council of Europe, Committee of Experts on Air Pollution, *First European Congress, Influence Air Pollution, Plants and Animals,* Wageningen, Netherlands.

Horvat, T., Vidaković-Cifrek, Ž., Oreščanin, V., Tkalec, M. & Pevalek-Kozlina, B. (2007). Toxicity assessment of heavy metal mixtures by *Lemna minor* L. *Science of the Total Environment*, 384(1-3), 229–238.

Hoshina, M. M. (2002). Avaliação da possí vel contaminação das águas do Ribeiro Claro -município de Rio Claro, pertencente á bacia do Rio Corumbataí, por meio de testes de mutagenicidade em *Allium cepa*, trabalho de conclusão (Bacharel e Licenciatura - Ciências Biolo´ gicas), Universidade Estadual Paulista, Rio Claro/SP.

Hossain, M. K., Strezov, V., Chan, K. Y., Nelson, P. F. (2010). Agronomic properties of wastewater sludge biochar and bioavailability of metals in production of cherry tomato (*Lycopersicon esculentum*). *Chemosphere*, 78(9), 1167-1171.

Ichikawa, S. & Sparrow, A. H. (1969). Analyses of radiation-induced loss of reproductive integrity in *Tradescantia* stamen hairs, an essentially single meristematic cell system. *The Japanese Journal of Genetics,* 44, 23-24.

Ichikawa, S. & Takahashi, C. S. (1978). Somatic mutations in *Tradescantia* stamen hairs exposed to ethyl methanesulfonaie. *Environmental and Experimental Botany*, 18, 19-25.

Ichikawa, S., Kanai, H., & Harada, H. (1990). Somatic mutation frequencies in *Tradescantia* stamen hairs treated with aqueous solutions of ethyl methanesulfonate and methyl methanesulfonate. *Japanese Journal of Genetics*, 65, 309.

Isidori, M., Ferrara, M., Lavorgna, M., Nardelli, A. & Parrella, A. (2003). In situ monitoring of urban air in Southern Italy with the *Tradescantia* micronucleus bioassay and semi-permeable membrane devices (SPMDs). *Chemosphere*, 52, 121-126.

ISO/CD 20079 (2001). *Water quality*- Determination of the Toxic effect of water Constituents and Waste water to Duckweed (*Lemna minor*) - Duckweed growth inhibition test. [Online] Available:
http://www.lemnatec.com/faq/standards-faq

ISO 11269-1 (2012) *Soil quality* - Determination of the effects of pollutants on soil flora. Part 1: Method for the measurement of inhibition of root growth. [Online] Available:
http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=19247

Jain, A. K. & Sarbhoy, R. K. (1987). Cytogenitical studies on the effects of some chlorinated pesticides. Effect on somatic chromosomes of *Lens* and *Pisum*. *Cytologia*, 52, 47-53.

Jain, S. K., Vasudevan, P. & Jha, N. K. (1989). Removal of some heavy metals from polluted water by aquatic plants: Studies on duckweed and water velvet. *Biological Wastes*, 28, 115-126.

Jastrebova, O., Kovalchuk, I. & Kovalchuk, L. (2010). Analyzes of soil genotoxicity in the city of Ivano-Frankivsk using *Nicotiana tabacum* SU/+ plants. *Advances in Bioresearch,* 1(1), 199-204.

Jha, A. M. & Singh, C. (1994). Clastogenicity of lanthanides - induction of micronuclei in root tips of *Vicia faba*. *Mutation Research/Genetic Toxicology*, 322(3), 169-172.

Jiang, W., Liu, D. & Liu, X. (2001). Effects of copper on root growth, cell division, and nucleolus of *Zea mays*. *Biologia Plantarum,* 44, 105-109.

Kanaya, N. Gill, B. S., Grover, I. S., Murin, A., Osiecka, R., Sandhu, S. S. & Andersson H. C. (1994). *Vicia faba* chromosomal aberration assay *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 310(2), 231-247.

Kaul, B. L. (1969). The production of chromosomal aberrations by propanesultone in *Vicia faba.*
Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 7(3), 339-347.

de Kergommeaux, D. J., Grant, W.F. & Sandhu S.S. (1983). Clastogenic and physiological response of chromosomes to nine pesticides in the *Vicia faba* in vivo root tip assay system. *Mutation Research/Genetic Toxicology*, 124(1), 69-84.

Khellaf, N. & Zerdaoui, M. (2009). Growth response of the duckweed *L. minor* to heavy metals pollution. *Iranian Journal of Environmental Health Science & Engineering,* 6(3), 161-166.

Kihlman, B. A. (1975). Root tips of *Vicia faba* for the study of the induction of chromosomal aberrations. *Mutation Research*, 31, 401-402,

Kopliku, D. Mesi A. & Golemi, S. (2011). Genotoxicity screening of flooded agricultural areas water sources in NënShkodra lowland using *Allium cepa* chromosome aberration assay. *Proceedings book of ICAFE*, Korçë, Albania, 27-28 May, 221-229.

Kopliku, D. Mesi A. & Golemi, S. (2012). Cytotoxicity screening of some west side sampling points in Shkodra lake and Buna river using Allium test. BALWOIS 2012, Conference on Water, Climate & Environment, Ohrid, Macedonia [Online] Available:
http://ocs.balwois.com/index.php?conference=BALWOIS&schedConf=BW2012&page=schedConf&op=presentations

Kristen, U. (1997). Use of higher plants as screens for toxicity assessment. *Toxicology in Vitro*, 11, 181-191.

Kuglik, P., Veselska, R., & Relichova, J., (1994). Sensitivity of plant cytogenic and genetic shortterm assays for evaluating genetic damage induced by chemical mutagens. *Cell Biology International,* 18, 543.

Kungolos, A. G., Brebbia, C. A., Samaras, C. P. & Popov, V. (Eds.) (2006). *Environmental toxicology*. WIT Press, Southampton, UK.

Leme, D. M. & Marin-Morales, M. A. (2008).Chromosome aberration and micronucleus frequencies in *Allium cepa* cells exposed to petroleum polluted water-a case study. *Mutation Research*, 650, 80-86.

Levan, A. (1938). The effect of colchicines in root mitosis in *Allium*. *Hereditas,* 24, 471-486.

Levan, A. (1945). Cytological reactions induced by inorganic salt solutions. *Nature*, 156, 751-752.

Liu, D. H., Jiang, W. S., Wang, W. & Zhai, L. (1995). Evaluation of metal ion toxicity on root tip cells by the *Allium* test. *Israel Journal of Plant Sciences*, 43, 125-133.

Liu, W., Li, P., Qi, X. M., Zhou, Q., Zheng, L., Sun, T. & Yang, Y. (2005). DNA changes in barley (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD analysis. *Chemosphere,* 61, 158-167

Liu, D., Jiang, W., Meng, Q., Zou, J., Gu, J. & Zeng, M. (2009). Cytogenetical and ultrastructural effects of copper on root meristem cells of *Allium sativum* L. *Biocell* 33(1), 25-32.

Lopez-Millan, A. F., Sagardoy, R., Solanas, M., Abadia, A. & Abadia, J. (2009). Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in hydroponics. *Environmental and Experimental Botany*, 65 (2-3), 376-385.

Lower, W. R., Rose, P. & Drobney, K. (1978). *In situ* mutagenic and other effects associated with lead smelting. *Mutation Research*, 54, 83.

Lower, W. R., Underbrink, A. G., Yanders, A. F., Roberts, K., Ranney, T. K., Lombard. G. T., Hemphill, D. D. & Clevenger, T. (1984). New technology for assessing mutagenicity of water and water-related sediments. In: *Second International Conference on Groundwater Quality Research,*(pp. 194-196). Oklahoma State, USA.

Ma, T. H., Sparrow, A. H., Schairer, L. A. & Nauman, A. F. (1978). Effect of 1,2-dibromoeth-ane (DBE) on meiotic chromosomes of *Tradescantia.* *Mutation Research/Genetic Toxicology*, 58, 251-258.

Ma, T. & Grant, W. (1982). The Tradescantia - adventurous plants. *The herbarist*, 48, 36-44.

Ma, T. H., Lower, W. R., Harris, F. D., Poku, J., Anderson, V. A., Harris, M. M., & Bare, J. L., (1983b). Evaluation by the *Tradescantia*-micronucleus tests on the mutagenicity of internal combustion engine exhaust fumes from diesel and diesel-soybean oil mixed fuels. In: *Short-Term Bioassay in the Analysis of Complex Environmental Mixtures* (III, pp. 191-214). Waters, Sandhu, Lewias.

Ma, T. H. & Harris, M. M. (1985). *In situ* monitoring of environmental mutagens. In: Gorsuch/Dwyer/Ingersoll/Lapoint (Eds.), *Environmental toxicology and risk assessment.* ASTM STP 1216, Philadelphia, USA, 77-106.

Ma, T. H. & Harris, M. M. (1987a). *Tradescantia* micronucleus (Trad-MCN) assay - a potential indoor pollution monitor. *Environtal Mutagen*, 9(8), 65.

Ma, T. H. & Harris. M. M. (1987b). *Tradescantia* micronucleus (Trad-MCN) bioassay - a promising indoor air pollution monitoring system. In: *4th International Conference on Indoor Air Quality & Climat,* 1, 243-247.

Ma, T. H., Xu, Z., Xu, C., McConnell, H., Rabago, E. V., Arreola, G. A. &. Zhang, H (1995). The improved *Allium*/*Vicia* root tip micronucleus assay for clastogenicity of environmental pollutants. *Mutation Research*, 334, 185-195.

Ma, T. H. (1995). *Tradescantia* (spiderwort) plants as biomonitors of the genotoxicity of environmental pollutants. In: Butterworth, F. M., Corkum, L. D., & Guzman-Rincon, J. (Eds.), *Biomonitors and Biomarkers as Indicators of Environmental Change* (I, pp. 207-216). New York: Plenum Press.

Ma, T. H., Xu, C., Liao, S., McConnell, H., Jeong, B. S., & Won, C. D., (1996). *In situ* monitoring with the *Tradescantia* bioassays on the genotoxicity of gaseous emissions from a closed landfill site and an incinerator. *Mutation Research*, 359, 39-52.

Mackenzie, S. M., Waite, S., Metcalfe, D. J. & Joyce, C. B. (2003). Landfill leachate ecotoxicity
experiments using *Lemna minor. Water, Air, and Soil Pollution Focus*, 3, 171-179.

MAFCP (2011). Albanian Agriculture 2011, Fact sheet. Ministry of Agriculture, Food and Consumer Protection. Albdesign. Tirane, Albania.

Meng, Q. M., Zou, J., Zou, J. H., Jiang, W. S., Liu, D. H. (2007). Effect of Cu^{2+} concentration on growth, antioxidant enzyme activity and malondialdehyde content in Garlic (*Allium sativum* L.). *Acta Biologica Cracoviensia Series Botanica,* 49, 95-101.

Mericle, L. W. & Mericle. R. P. (1971). Somatic mutations in clone 02 *Tradescantia.* A search for genetic identity. *Journal of Heredity,* 62, 323-328.

Mesi, A. & Kopliku, D. (2011). *Allium* test of root growth for toxicity assessment as a standard in environmental monitoring of some aqueous sources of Malësia e Madhe. *Journal of International Environmental Application & Science*, 6(5), 654-660.

Mesi, A., Kopliku, D. & Golemi, S. (2011). The *Allium* test - an alternative in environmental water monitoring of Shkodra lake, Buna and Drini rivers. Proceedings Book of International Conference Alblakes II, Elbasan, Albania, 217-223.

Mesi, A., Kopliku, D. & Golemi, S. (2012). Correlative evaluation between experimental hexavalent chromium ion concentrations and root length of *Allium cepa (L.)* in some riverside points of NënShkodra Lowland. BALWOIS 2012, Conference on Water, Climate & Environment, Ohrid, Macedonia [Online] Available:
http://ocs.balwois.com/index.php?conference=BALWOIS&schedConf=BW2012&page=schedConf&op=presentations

Miadoková, E., Vlčková, V., Dúhová, V., Trebatická, M., Garajová, Ĺ., Grolmus, J., Podstavková, S. & Vlček D. (1992). Effects of supercypermethrin, a synthetic developmental pyrethroid, on four biological test systems. *Mutation Research/Genetic Toxicology*, 280(3), 161-168.

Miretzky, P., Saralegui, A. & Cirelli, A. F. (2004). Aquatic macrophytes potential for the simultaneous removal of heavy metals (Buenos Aires, Argentina). *Chemosphere*, 57, 997-1005.

Mišík, M., Ma, T. H., Nersesyan, A., Monarca, S., Kim J. K. & Knasmueller S. (2010). Micronucleus assays with *Tradescantia* pollen tetrads: an update. *Mutagenesis, 26 (1*), 215-221.

Monarca, S., Feretti, D., Zanardini, A., Falistocco, E. & Nardi, G. (1999). Monitoring of mutagens in urban air samples. *Mutation Research*, 426, 189-192.

Nasto, Th. & Bardhi, N. (2004). Bazat e perimtarisë dhe prodhimit të perimeve, (pp. 427). *Dita 2000*, Tiranë, Albania.

Nauman, C. H., Sparrow, A. H. & Schairer, L. A. (1976). Comparative effects of ionizing radiation and two gaseous chemical mutagens on somatic mutation induction in one mutable and two non-mutable clones of *Tradescantia. Mutation Research*, 38, 53-70.

NEA (2000). State of the Environment REPORT, 1997–1998. *National Environmental Agency*, Tirana, Albania.

Nayar, G. G. & Sparrow, A. H. (1967). Radiation-induced somatic mutations and the loss of reproductive integrity in *Tradescantia* stamen hairs. *Radiation Botany,* 7, 257-267.

OECD (2000). Guidance document on aquatic toxicity testing of difficult substances and mixtures. *OECD Environmental Health and Safety Publications Series on Testing and Assessment,* 23, Paris, France.

Nilan, R. A. (1978). Potential of plant genetic systems for monitoring and screening mutagens.

Environmental Health Perspectives, 27, 181–196. Oguntimehin, I., Eissa, F. & Sakugawa, H. (2010). Negative effects of fluoranthene on the ecophysiology of tomato plants (*Lycopersicon esculentum* Mill.) Fluoranthene mists negatively affected tomato plants. *Chemosphere*, 78 (7), 877-884.

Opeolu, B. O., Adenuga, O. O., Ndakidemi, P. A. & Olujimi, O. O. (2010). Assessment of phyto-toxicity potential of lead on tomato (*Lycopersicon esculentum* L.) planted on contaminated soils. *International Journal of Physical Sciences,* 5 (2), 68-73.

Öztürk Çali, I. (2009). Cytogenetic effects of fungicide applications of tomato (*Lycopersicon esculentum* Mill.). *Turkish Journal of Biology*, 33, 205-209.

Palacio, S. M., Espinoza-Quiñones, F. R. Galante, R. M. Zenatti, D. C., Seolatto, A. A., Lorenz, E. K., Zacarkim, C. E., Rossi, N., Rizzutto M. A. & Tabacniks, M. H. (2005). Correlation between heavy metal íons (Copper, Zinc, Lead) concentrations and root length of *Allium cepa* L. in polluted river water. *Brazilian Archives of Biology and Technology*, 48, 191-196

Plewa, M. J. (1978). Activation of chemicals into mutagens by green plants: A preliminary discussion. *Environmental Health Perspectives*, 27, 45.

Plewa, M. J. (1982). Specific-locus mutation assay in *Zea may*s: A report of the U.S. environmental protection agency Gene-Tox program. *Mutation Research/Reviews in Genetic Toxicology*, 99 (3), 317-337.

Poma, A., Arrizza, L., Picozzi, P. & Spanò, L. (2002). Monitoring urban air particulate matter (fractions PM 2.5 and PM 10) genotoxicity by plant systems and human cells in vitro: A comparative analysis. *Teratogenesis, carcinogenesis, and mutagenesis*, 22 (4), 271-84.

Poschenrieder, C., Gunsé, B. & Barceló, J. (1989). Influence of cadmium on water relations, stomatal resitance, and abscisic acid content in expanding bean leaves. *Plant Physiology*. 90, 1465-1371.

Radić, S., Stipaničev, D., Vujčić, V., Rajčić, M. M., Širac, S. & Pevalek-Kozlina, B. (2010). The evaluation of surface and wastewater genotoxicity using the *Allium cepa* test. *Science of the Total Environment*, 408, 1228-1233

Rank, J. & Nielsen, M. H. (1993). A modified *Allium* test as a tool in the screening of the genotoxicity of complex mixtures. *Hereditas*, 18, 49-53.

Rank, J. (2003). The method of Allium anaphase-telophase chromosome aberration assay. *Ekologija*, 38-42.

Ribas, A., Filella, I., Gimeno, B.S. & Penuelas, J. (1998). Evaluation of tobacco cultivars as bioindicators and biomonitors of ozone phytotoxical levels in Catalonia (N.E. Spain). *Water, Air and Soil Pollution,* 197, 347-365.

Ribeiro, D. M. F. & Salvadori, E. K. (2003). In: Marques (Eds.), *Mutagênese Ambiental* (pp. 201-219). Ulbra, Canoas.

Ribeiro, L.R. (2006). Teste do micronúcleo em medula óssea de roedores in vivo. *Sciences of Total Environment*, 363, 114.

Rodrigues, G. S., Ma, T. H., Pimentel, D. & Weinstein, L. H. (1997). *Tradescantia* bioassays as monitoring systems for environmental mutagenesis: a review. *Critical Reviews in Plant Sciences*, 16, 325-359.

Sadowska, A., Pluygers, E., Narkiewicz, M., Pawelczak, A. & Lata, B. (1994). Environmental genotoxicity and cancer risk in humans: a combined evaluation correlating the results of the *Tradescantia* micronucleus assay in the field and human biomarker assessments in serum. The TRAD-MCN assay. *European Journal of Cancer Prevention*, 3, 69-78.

Salam, M. A., Siddique, M. A., Rahim, M. A., Rahman, M. A. & Saha, M. G. (2010). Quality of tomato (*Lycopersicon esculentum* Mill.) as influenced by boron and zinc under different levels of NPK fertilizers. *Bangladesh Journal of Agricultural Research*, 35 (3), 475-488.

Sanda-Kamigawara, M., Ichikawa, S., & Watanabe, K. (1991). Spontaneous, radiation-and EMS induced somatic pink mutation frequencies in the stamen hair and petals of a diploid clone of *Tradescantia* KU 27. *Environmental and Experimental Botany*, 31, 413-421.

Sang, N. & Li, G. (2004). Genotoxicity of municipal landfill leachate on root tips of *Vicia faba*. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 560(2), 159-165.

Sang, N., Han, M., Li, G. & Huang, M. (2010). Landfill leachate affects metabolic responses of *Zea mays* L. seedlings. *Waste Management*, 30 (5), 856-62.

Sant'Anna, S. M. R., Esposito, M. P., Domingos, M. & Souza, S. R. (2008). Suitability of *Nicotiana tabacum* Bel W3 for biomonitoring ozone in São Paulo, Brazil. *Environmental Pollution*, 151(2), 389-394.

Schairer, L. A., Van't Hof, J., Haves, C. C., Bunon, M. R. & de Serres, F. J. (1979). Measurement of biological activity of ambient air mixtures using a mobile laboratory for *in situ* exposure, preliminary results from the *Tradescantia* plant test system. In: Sandhu, S. S. & Claxton (Eds.), *Application of Short-term Bio assays in the Fractionation and Analysis of Complex Environmental Mixtures* (pp. 419-440). Plenum Press, New York.

Shima, N. & Ichikawa, S. (1994). Synergism detected among methyl methanesulfonate, ethyl methanesulfonate and X-rays in inducing somatic mutations in the stamen hairs of *Tradescantia* clone BNL 4430. *Environmental and Experimental Botany,* 34, 393-408.

Shima, N., & Ichikawa, S. (1995). Mutagenic synergism detected between dimethyl sulfate and

X-rays but not found between N-methyl-N-nitrosourea and X-rays in the stamen hairs of *Tradescantia* clone BNL 4430. *Mutation Research,* 331, 79-87.

Schmid, W. (1975). The micronucleus test. Mutation Research, 31(1), 9-15.

Smaka-Kincl, V., Stegnar, P., Lovka, M., Toman, M. J. (1996). The evaluation of waste, surface and ground water quality using the *Allium* test procedure. *Mutation Research*, 368, 171-179.

Souguir, D., Ferjani, E., Ledoigt, G. & Goupil, P. (2008). Exposure of *Vicia faba* and *Pisum sativum* to copper-induced genotoxicity. *Protoplazma*, 233(3-4), 203-207.

Souza, T. S., Hencklein, F. A., Angelis, D. F., Gonçalves, R. A. & Fontanetti, C. S. (2009). The *Allium cepa* bioassay to evaluate land-farming soil, before and after the addition of rice hulls to accelerate organic pollutants biodegradation. *Ecotoxicology Environmental Safety,* 72(5), 1363-8.

Srivastava, R., Kumar, D. & Gupta, S. K. (2005). Bioremediation of municipal sludge by vermitechnology & toxicity assessment by *Allium cepa*. *Bioresource Technology*, 96, 1867-1871.

Srivastava, K. & Mishra, K. K. (2009). Cytogenetic effects of commercially formulated atrazine on the somatic cells of *Allium cepa* & *Vicia faba*. *Biochemical Physiology*, 93, 8-12.

SSC & REC/CEE (2000). Strategic environmental analysis of Albania, Bosnia & Herzegovina, Kosovo and Macedonia. Final Report. *Scandiaconsult Natura AB and The Regional Environmental Center for Central and Eastern Europe*.

Sumer Aras, S., Aydin, S. S., Körpe, D. A. & Dönmez, Ç. (2011). Comparative Genotoxicity Analysis of Heavy Metal Contamination in Higher Plants. *Ecotoxicology*, 6, 107-124.

Tano, S. (1989). *In situ* detection of induced mutations with chemicals by *Tradescantia. Environmental and Molecular Mutagenesis,* 14(15), 197.

Taraldsen, J. E. & Norberg-King, T. J. (1990). New method for determining effluent toxicity using duckweed (*Lemna Minor*). *Environmental Toxicology and Chemistry*, 9 (6), 761-767.

Tempelaar, M. J., de Both, M. T. J. & Versteegh J. E. *G.* (1982). Measurement of SCE frequencies in plants: a simple Feulgen-staining procedure for *Vicia faba. Mutation Research*, 103, 321-326.

Tort, N., Öztürk, İ. & Güvensen, A. (2005). Effects of some fungicides on pollen morphology and anatomy of tomato (*Lycopersicon esculentum* Mill.). *Pakistan Journal of Botany*, 37 (1), 23-30.

UN/ECE (2000). Guidelines on Monitoring and Assessment of Transboundary Rivers. Vetayasuporn, S. (2006). Effects of biological and chemical fertilizers on growth and yield of shallot (*Allium cepa var. ascolonicum*) production. *Journal of Biological Sciences*, 6(1), 82-86.

UNEP (2000). Post-Conflict Environmental Assessment-Albania. UNEP, Switzerland.

U.S. EPA (1985). *Lemna* Acute Toxicity Test. *Federal Register*, Vol. 50 (797.1160), 188 (1), 39331-39333.

Usha, M., Pranav, K., Varshney, C. K., Mina, U. & Kumar, P. (2010). Effect of ozone exposure on growth, yield and isoprene emission from tomato (*Lycopersicon esculentum* L.) plants. *Vegetable Crops Research Bulletin*, 72, 35-48.

Wadhia, K. & Thompson, K. C. (2007). Low-cost ecotoxicity testing of environmental samples using microbiotests for potential implementation of the Water Framework Directive. *Trends* in Analytical Chemistry, 26, 300.

Wang, W. (1987). Toxicity of nickel to common duckweed (*Lemna minor*). *Environmental Toxicology and Chemistry,* 6(12), 961-967.

Wang, W. (1990). Literature review on duckweed toxicity testing, *Environmental Research,* 52(1), 7-22.

Waters, M. D. & Auletta, A. (1981). The GENE-TOX Program: genetic activity evaluation. *Journal of Chemical Information & Computer Sciences*, 21(1), 35-38.

Wolska, L., Sagajdakow, A., Kuczynska, A. & Namiesnik, J. (2007). Application of ecotoxicological studies in integrated environmental monitoring. Possibilities and problems. *Trends in Analytical Chemistry*, 26, 332.

Wolska, L., Namiesnik, J., Michalska, M. & Bartoszewicz, M. (2008). Preliminary Study on Toxicity of Aquatic Ecosystems in Bug River Basin. *Polish Joiurnal of Environment Studies*, 17(5), 811-816.

Yildiz, M. & Arikan, E. S. (2008). Genotoxicity testing of quizalofop-P-ethyl herbicide using the *Allium cepa* anaphase-telophase chromosome aberration assay. *Caryologia*, 61(1), 45-52.

Xing, W. J. & Zhang, Z. L. (1990). A comparison of SCE test in human lymphocytes and *Vicia faba*: a hopeful technique using plants to detect mutagens and carcinogens. *Mutation Research*, 241(2), 109-13.

Xhuveli, L., Çekani, N., Papajani, M, Memajdini, Q., Petrela, I., Nesturi, D., Shundi, A. & Xhepa, S. (1987). Fitoteknia (pp-749). *Mihal Duri*, Tiranë, Albania.

Zayed, A., Gowthaman, S. & Terry, N. (1998). Phytoaccumulation of trace elements by wetland plants: I- Duckweed. *Journal of Environmental Quality*, 27, 715-721.

Zhang, Z., Yang, J., Zhang, Q. & Cao, X. (1991). Studies on the utilization of a plant SCE test in detecting potential mutagenic agents. *Mutation Research/Genetic Toxicology*, 261(1), 69-73.

Žegura, B., Heath, E., Černoša, A. & Filipič, M. (2009). Combination of in vitro bioassays for the determination of cytotoxic and genotoxic potential of wastewater, surface water and drinking water samples. *Chemosphere*, 75, 1453-60.

Zhang, S., Zhang, H., Qin, R., Jiang, W. & Liu, D. (2009). Cadmium induction of lipid peroxidation and effects on root tip cells and antioxidant enzyme activities in *Vicia faba* L. *Ecotoxicology*, 18, 814-823.