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www.wastereuse.eu

Action 2 - Initial assessment of existing AW treatment technologies

Deliverable "Assessment of potential adverse effects (phyto-toxicity, contamination of soils and groundwater)"

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Executive summary

In the line of Action 2, all available data regarding funded projects focused on the development/ application of technologies for the treatment of agricultural wastes (AW) produced in large quantities in the Mediterranean region, such as olive oil mill wastes (OMW), wine, animal waste and various other AW, have been collected by Technical University of Crete (TUC), through an extensive search of relevant and available databases (LIFE, Sciencedirect, Scopus, Cordis, Google etc.) A total of 49 funded projects have been identified; 14 of the projects are ongoing and active websites are available for 32 projects.

All available projects have been included in a comprehensive inventory which is uploaded on the web-site of the WasteReuse. The technologies were also quantitatively evaluated using weighted technical, environmental, economical and socio-cultural indicators. Best AW treatment technologies were selected and proposed to responsible Beneficiaries of Actions 3 and 4 (CEBAS-CSIC, CCIAA and CERSAA) for consideration.

In the present deliverable, the potential adverse effects of the disposal of the most important AW produced in the Med region, on soils and water bodies are discussed. For instance, toxicity is a very significant parameter for the characterization of AW and it should be taken into account before and after treatment to a) select the most appropriate treatment technologies which should reduce the toxicity of treated AW to acceptable levels, b) define the use of the final products and c) define the optimum management strategy of the secondary wastes produced in order to eliminate adverse impacts on humans and environment. Numerous toxicity tests for wastes, soil and water are used to assess the concentration of the contaminants in leachates and compare with thresholds as well as to evaluate the hazard of the contaminants to particular test organisms. Composting of AW is the most commonly used management option and the final product can be used as soil improver to enhance crop production and minimize risk for soil, water and ecosystems.

The contents of the deliverable are:

1. Introduction

In the line of Action 2, all available data regarding funded projects focused on the development/ application of technologies for the treatment of agricultural wastes (AW) produced/applied in Med countries and mainly in Spain, Italy and Greece, have been collected by Technical University of Crete (TUC). All available projects funded so far by European Commission (especially LIFE) as well as projects developed by private funding and aiming to recover useful by-products, minimize environmental impacts as well as produce “cleaner” waste for safe disposal have been included in a comprehensive inventory which has been uploaded on the web-site of WasteReuse. The technologies were also quantitatively evaluated using weighted technical, environmental, economical and socio-cultural indicators.

The outcomes of Action 2 are being considered in the lab experiments in the line of Actions 3 and 4 (in progress) to evaluate the treated wastes derived from the application of different technologies developed so far, regarding their suitability to improve crop production and quality as well as to assess the potential effects on soil properties. The most suitable, environment friendly, low cost technologies will be used for the development of alternative cultivation practices for the main water and nutrient consuming crops in Spain and Italy; the feasibility of the application of treated wastes in open field and greenhouse cultivations will be also demonstrated (Actions 5 and 6).

In the present deliverable, the potential adverse effects of the disposal of the most important AW produced in the Med region, on soils and water bodies are discussed. For instance, toxicity is a very significant parameter for the characterization of AW and it should be taken into account before and after treatment to a) select the most appropriate treatment technologies which should reduce the toxicity of treated AW to acceptable levels, b) define the use of the final products and c) define the optimum management strategy of the secondary wastes produced in order to eliminate adverse impacts on humans and environment. Numerous toxicity tests for wastes, soil and water are used to assess the concentration of the contaminants in leachates and compare with thresholds as well as to evaluate the hazard of the contaminants to particular test organisms. Composting of AW is the most commonly used management option and the final product can be used as soil improver to enhance crop production and minimize risk for soil, water and ecosystems.

2. AW production and adverse effects

The most important AW produced in the Med region include olive oil mill wastes (OMW), wine, swine and other animal waste, rice straw and various other AW (such as waste from handling of fruits and vegetables, horse or chicken manure, wheat straw etc). AW can be in the form of solid, liquid or slurries depending on the nature of agricultural activities are mainly characterized by seasonal production and should be rapidly removed from the field to avoid interferences with other agricultural activities (Sarmah, 2009). Depending on the agricultural activity, AW can be categorized as in Table 1 (Loehr, 1978).

Although the volume of wastes produced by the agricultural sector is significantly lower compared to wastes produced by other sectors, their pollution potential is usually very high. For example, AW may be characterized as potentially hazardous and toxic when disposed untreated on soil or in water bodies due to their high content of recalcitrant compounds. Application of AW such as manure on crop land and pasture can result in decrease in soil permeability and also adversely affect crop growth due to inhibitory amounts of nitrite nitrogen (NO_2-N) or salts added in soil. Excess loading of nitrogen and phosphorus from AW applied on land may cause eutrophication of water bodies or contamination of drinking water (Sharpley et al., 1984; Anderson et al., 2002).

Table 1. Characterization of AW depending on the agricultural activity (Loehr, 1978)

Agricultural activity	Wastes	Method of disposal/treatment, by-products
Crop production and harvesting	Straw, stover	Land application, burning, plowing
Fruit and vegetable processing	Biological sludges, trimmings, peels, leaves, stems, soil, seeds and pits	Landfilling, animal feed, land application, burning
Sugar processing	Biological sludges, pulp, lime mud	Landfilling, burning, composting, animal feed
Animal production	Blood, bones, feather, litter, manures, liquid effluents	Land application, fertilizer
Dairy product processing	Biological sludges	Landfilling, land spreading
Leather tanning	Fleshings, hair, raw and tanned trimmings, lime and chrome sludge, grease	By-product recovery, landfilling, land spreading
Rice production	Bran, straw, hull	Mulch/soil conditioner, packaging material for glass and ceramics
Coconut production	Stover, cobs, husk, leaves, coco meal	Vinegar, activated carbon, coir products

2.1 OMW

The annual OMW production in the Mediterranean countries is estimated to be over $3x10^7$ m^3. OMW is easily fermentable, dark and turbid liquid and its characteristics vary depending on the extraction process, the olive variety, the soil and climatic conditions and the cultivation method. The OMW contamination potential is related to its high organic load (BOD_5: 20-120 g/L; COD: 40-240 g/L), high content of phenolic compounds and significant concentration of magnesium, potassium and phosphate salts. In addition, it contains several organic compounds such as lipids, sugars, organic acids, tannins, pectins and lignins. It is also characterized by rather low pH (4.5-6) and high electrical conductivity (3-22 mS/cm) (Alburquerque et al., 2004; Davies et al., 2004; D' Annibale et al., 2004; De Marco et al., 2007; EC DG Environment, 2010).

Due to the scattering of small olive oil production units in the Mediterranean countries, evaporation in lagoons and disposal on agricultural land are the most commonly used OMW management options. Uncontrolled disposal of OMW may affect soil acidity, salinity, N immobilization, microbial activity, nutrient leaching, lipids concentration, soil hydrophobicity, water retention capacity, infiltration rates and cause strong phytotoxic effects. The phytotoxic effect of OMW is mainly associated with their high concentration of phenols (catechol, hydroxytyrosol, tyrosol, oleuropein) that are known to inhibit plant and bacterial growth (Abu-Zreig and Al-Widyan, 2002; Sierra at al., 2007).

Discharge of OMW, even diluted, in streams, rivers and other water bodies may severely affect ecosystems and reduce the potential of self-purification mechanisms necessitating thus the application of monitoring schemes to assess spatial and temporal effects (Karaouzas et al., 2011a; Danellakis et al., 2011).

Although in most countries no specific guidelines exist, studies have shown that when OMW are used as soil amendment, no more than 50 m^3 OMW per ha should be spread in a single application (Defra, 2009). It has been reported that continuous uncontrolled application of OMW may cause surface sealing, affect soil properties and contribute to eutrophication of freshwaters (S'habou et al., 2009; Kavvadias et al., 2011). Soil acidity may be also increased and can be neutralized only in cases the content of soil carbonates is high. High OMW application rates affect also the levels of exchangeable K and the content of $N\text{-}NO_3$ in soils (Paredes et al., 2005). Another major environmental concern is associated with P accumulation in soil and the long period required, up to 20 years, so that P content reaches again acceptable levels for agronomic use (López-Piñeiro et al., 2008).

Application of OMW on soils in high rates may severely affect soil properties, plant growth and groundwater quality. Apart from pre-treatment, which due to the wide scattering of mills in all Mediterranean countries should be better considered at mill scale, other important factors such as optimum OMW application rates, frequency of application, soil types, types of cultivated plants and depth of aquifer should be taken into account prior to application. Specific care should be taken if OMW are applied on sandy soils or sensitive water bodies.

Various treatment options aiming to decontaminate OMW prior to discharge, reuse or safe disposal have been investigated and some of them have been applied at mill scale to mitigate environmental impacts. These treatment options involve a) physical, eg. filtration, centrifugation, dissolved-air flotation; b) physico-chemical, eg. neutralization/coagulation, electrochemical oxidation; c) biological, eg. under anaerobic or aerobic conditions and d) thermal methods, eg. combustion/pyrolysis and lagooning (Paraskeva and Diamantopoulos, 2006; Mekki et al., 2007). Among them, co-composting of OMW with other AW may result in products with low phytotoxicity or high horticultural value which may be used as soil additives. This technique is advantageous compared to land application of raw OMW, which cause high risk for soil and water contamination (Papafotiou et al., 2005; Aviani et al., 2010). Prior to the selection and use of a management option, protection of soil and water quality as well as human health should be always considered as a matter of high importance (Karlen et al., 1997; Doran and Zeiss, 2000).

2.2 Wine waste

Wine production requires a considerable amount of resources such as water, fertilizers and organic amendments producing also large amounts of solid wastes and wastewater during a short period of the year. Solid wastes include grape stalks, grape marc and wine lees and can contaminate soil and water resources. Wastewater has a pH of 3.5 to 7, high organic load (4-7 g/L BOD and up to 10 g/L COD), high salinity (3-4 mS/cm) and high content in sulphide compounds which may lead to odour problems and eutrophication of water sources due to nitrogen concentration (Report of LIFE03 ENV/GR/000223 project, 2004; Bustamante et al., 2008).

In order to minimize the environmental impacts, main management options involve composting of wine residues and application on land as organic fertilizer enhancing plant growth (Lo Curto and Tripodo, 2001; Louli et al., 2004). Also wine waste can be treated properly to produce or recover various by-products including alcohol and tartrates from grape marc and wine lees, yeast from grape marc, phenolic compounds and grape seed oil (Díaz et al., 2002; Madejón et al., 2002; Bertrán et al., 2004).

2.3 Animal waste

Animal wastes include mostly manures, poultries and slaughterhouse waste. They can threaten surface- and groundwater quality in case of waste spills, leakage from waste storage facilities and runoff from fields where an excessive amount of waste has been applied as fertilizer. Dust emission problems eg. during the unloading of poultry as well as offensive odours from slurries, lairages and inedible offal storage, are also reported.

The swine industry produces wastes in huge quantities and direct disposal causes severe environmental impacts such as acidification due to emissions of NH_3, SO_2 and NO_x, increase of greenhouse effect due to emissions of CO_2, CH_4 and N_2O, increase of organic and nutrient loading to surface- and groundwater due to the high BOD and nutrient content of piggery effluent, diffuse spreading of heavy metals etc. Livestock wastes also contain significant amounts of steroid hormones (naturally released by animals of all species in urine) that may cause adverse effects on terrestrial and aquatic organisms (Jobling et al., 1998; Boxall et al., 2004). Commonly employed waste treatment systems include aerobic and anaerobic ponds, anaerobic digestion, aerobic biological treatment using continuous flow activated sludge systems or sequencing batch reactors, composting of solid manure, incineration etc. (http://pigwasteman.ari.gov.cy/).

Slaughterhouse wastes were treated in the past into meat- and bone-flour and industrial fat and used for animal feeding with high biological value. However, the use of animal proteins for feeding was restricted due to bovine spongiform encephalopathy (BSE) risk. Their uncontrolled disposal can cause significant environmental problems, while another issue of concern is the high water consumption, which should follow EU legislation (fresh, potable water should be used for almost all washing operations and limited recycled water is used within the slaughterhouse) (Lóki et al., 2010).

The most common animal waste management practices include (a) cementing and curbing of animal confinement areas or planting of grassed buffers around these areas to limit waste runoff, (b) scraping or flushing systems and storage tanks or retention ponds to collect and store waste and (c) production of compost or organic fertilizer or additive to animal feed, to alternatively utilize waste.

2.4 Various other AW

Various wastes such as rice straw, leafs, agroindustrial and municipal solid wastes have been used for the production of compost which can be applied on soil and improve yield of plants (García-Gomez et al., 2002; Soumaré et al., 2003; Sie et al., 2011). Addition of sewage sludge has shown to improve, in most cases, the physical and chemical properties of soil due to its nitrogen, phosphorous and trace element content and therefore has been widely used for land application in many countries. However, heavy metals and pathogens may be accumulated in soil after long-term land application of sewage sludge; this adverse effect may be limited by increasing the pH of sewage sludge above 11 when mixed with lime (Debosz et al., 2002; Stabnikova et al., 2005).

3. AW treatment technologies

So far, many projects aiming at the development of AW treatment technologies have been funded within European funding schemes and especially LIFE, as seen in Table 2. A total of 49 funded projects have been identified by TUC search through all relevant and available databases (LIFE, Sciencedirect, Scopus, Cordis, Google etc.); 14 of the projects are ongoing, while active websites are available for 32 projects. All projects have focused on the development of innovative technologies for AW treatment as well as on the recovery of useful by-products and energy, minimization of the environmental adverse impacts and production of “cleaner” wastes for safe disposal.

All available technologies for AW treatment have been included in a comprehensive inventory which has been discussed in detail in the deliverable entitled “Inventory of all available technologies for AW treatment, grouped by level of development (lab, pilot, full scale)” in the frame of Action 2, WasteReuse project; the inventory has been also uploaded on the web-site of the project (www.wastereuse.eu). Details for each project (duration, funding scheme, budget, beneficiaries) as well as a short description of each developed technology are also included. More details can be found on the websites of the projects, where available.

All these technologies were evaluated based on available data and according to selected technical, environmental, economical and socio-cultural indicators, using a scale between 1 and 3. Four different scenarios were also considered and the best AW treatment technologies were selected for each waste and are being considered by the responsible Beneficiaries of Actions 3 and 4 (CEBAS-CSIC, CCIAA and CERSAA) for the evaluation of treated and untreated waste to be used in lab experiments. Thereafter the suitability of the wastes to improve crop production and quality as well as the identification of the potential effects on soil properties, will be assessed (Development of weight based indicators for quantitative evaluation of AW treatment technologies, Deliverable in the line of Action 2, WasteReuse, 2012).

Table 2. Number of funded projects per type of AW (by March 2012)

Waste	Number of funded projects (funding scheme)
Olive oil mill wastes (OMW)	20 (11 by LIFE, 3 by FP5, 3 so far by FP7, 1 by ERDF Innovative Actions 2000-2006, 1 by SME, 1 by FAIR)
Wine waste	4 (by LIFE)
Swine waste	7 (by LIFE)
Other animal waste	7 (6 by LIFE, 1 by FP7)
Rice straw	2 (by LIFE)
Various other AW	9 (by LIFE)

4. Toxicity tests and evaluation

Organic and inorganic contaminants contained in AW can adversely affect living organisms (humans, microbes, bacteria, vertebrates, aquatic organisms etc.) as well as the physical and chemical properties of the soil, water and plants. Toxicity tests are mainly used to assess the hazard of contaminants, singularly or in mixtures, to particular test organisms and are based on the measurable and progressive relationship between dose and effect under a set of given test conditions (Cal/EPA, 2009; Karaouzas et al., 2011b; Di Bene et al., 2012).

4.1 Waste toxicity

Leaching tests are used to estimate potential concentration of compounds that can leach from a solid waste. Typical leaching tests use a specified leaching fluid mixed with the solid waste for a specified time. Solids are then separated from the leaching solution which is tested for various contaminants. The type of leaching test performed can vary depending on the chemical, biological and physical characteristics of the waste or the environment in which the waste will be disposed.

A brief summary of the most commonly used leaching tests for the determination of toxicity of solid wastes (Extraction Procedure (EP) toxicity test, Toxicity Characteristic Leaching Procedure (TCLP) test, Synthetic Precipitation Leaching Procedure (SPLP) test, Multiple Extraction Procedure (MEP) test, California Waste Extraction Test (WET) and Shake Extraction of Solid

Waste with Water or Neutral Leaching Procedure) is provided in the following text. However, many other tests have been developed to estimate the potential toxicity of wastes, including:

- Ultrasonic Agitation Method for Accelerating Batch Leaching Test
- Extraction Procedure for Oily Waste
- Equilibrium Leach Test
- Static Leach Test Method
- Sequential Extraction Test

4.1.1 Extraction Procedure (EP) toxicity test

EP test (designated as EPA Method 1310) is used to determine whether a waste (liquid, solid or multiphase) exhibits the characteristic of extraction procedure toxicity. EP test may also be used to simulate the leaching of a waste disposed of in a sanitary landfill. For the EP test, maximum concentration values have been determined for 8 metals, 4 pesticides and 2 herbicides in an effort to establish regulations to prevent groundwater contamination. In laboratory, the solid waste is mixed with deinonized water in a ratio of 1:16 and agitated for a period of 24 hours. During agitation the pH of the solution is maintained at 5±0.2 and the temperature at 20-40 ^{o}C. Thereafter S/L separation takes place and liquid is analyzed (EP, 1990).

4.1.2 Toxicity Characteristic Leaching Procedure (TCLP) test

TCLP test (designated as EPA Method 1311) is used to determine the mobility of both organic and inorganic compounds in liquid, solid and multi phase wastes. It evaluates the leaching of metals, volatile and semi-volatile organic compounds and pesticides from wastes and is used to classify waste (hazardous or non hazardous) as suitable or not to be disposed in landfills.

The solids are extracted with an acetate buffer solution. An S/L ratio of 1/20 by weight is used for an extraction period of 18± 2 hours. After extraction, S/L separation takes place and the liquid extract is combined with any original liquid fraction of the wastes. Analysis is then conducted on the liquid filtrate/leachate to determine the compounds concentrations. In Table 3 threshold concentration of compounds in leachates produced according to TCLP test, are seen. If a solid waste fails the test for one or more of these compounds the waste is considered as hazardous (TCLP, 1990).

Although the TCLP is the most commonly used leachate test for estimating the actual leaching potential of wastes, it may not be applied in all conditions and for all types of wastes. For example the TCLP is not likely to be the optimal method in a landfill with alkaline conditions or for analyzing oily wastes.

4.1.3 Synthetic Precipitation Leaching Procedure (SPLP) test

SPLP test (designated as EPA Method 1312) is designed to determine the mobility of both organic and inorganic compounds in liquid, soils and waste as well as assess the leaching potential of soils, waste and wastewater under conditions simulating acid rain. The extraction fluid employed is a function of the region of the country where the soil sample is located. The SPLP experimentation is very similar to the TCLP; the main difference relies on the extracting fluid used which simulates acid rain conditions. SPLP may be used for solid wastes that have already been characterized as toxic according to TCLP test (www.epa.gov/industrialwaste).

4.1.4 Multiple Extraction Procedure (MEP) test

MEP test (designated as EPA Method 1320) was designed to simulate the leaching of a waste when acid rain precipitates repeatedly on a landfill and determine the highest concentration of each compound that is likely to leach out. The MEP can be used to evaluate liquid, solid and multiphase samples and is also very similar to the TCLP, while it is intended to simulate 1,000 years of freeze and thaw cycles and prolonged exposure to a leaching medium. One advantage of the MEP over the TCLP is that the MEP gradually removes excess alkalinity of the waste; thus the leaching behavior of metals can be evaluated as a function of decreased pH which increases the solubility of most metals (www.epa.gov/industrialwaste).

Table 3. Threshold concentration of compounds in leachates produced according to TCLP test

EPA number	Compound	Concentration, mg/L	EPA number	Compound	Concentration, mg/L
D004	Arsenic (As)	5	D032	Hexachlorobenzene	0.13
D005	Barium (Ba)	100	D033	Hexachlorobutadiene	0.5
D018	Benzene	0.5	D034	Hexachloroethane	3
D006	Cadmium (Cd)	1	D008	Lead (Pb)	5
D019	Carbon Tetrachloride	0.5	D013	Lindane	0.4
D020	Chlordane	0.03	D009	Mercury (Hg)	0.2
D021	Chlorobenzene	100	D014	Methoxychlor	10
D022	Chloroform	6	D035	Methyl ethyl ketone	200
D007	Chromium (Cr)	5	D036	Nitrobenzene	2
D023	o-Cresol	200	D037	Pentachlorophenol	100
D024	m-Cresol	200	D038	Pyridine	5
D025	p-Cresol	200	D010	Selenium (Se)	1
D026	Cresol	200	D011	Silver (Ag)	5
D016	2,4-D	10	D039	Tetrachloroethylene	0.7
D027	1,4-Dichlorobenzene	7.5	D015	Toxaphene	0.5
D028	1,2-Dichloroethane	0.5	D040	Trichloroethylene	0.5
D029	1,1-Dichloroethylene	0.7	D041	2,4,5-Trichlorophenol	400
D030	2,4-Dinitrotoluene	0.13	D042	2,4,6-Trichlorophenol	2
D012	Endrin	0.02	D017	2,4,5-TP (Silvex)	1
D031	Heptachlor	0.008	D043	Vinyl Chloride	0.2

4.1.5 California Waste Extraction Test (WET)

WET shall be used to determine extractable concentrations of toxic compounds in a waste or other material and is primarily used for inorganics, pesticides, herbicides, PCPs (pentachlorophenols) and PCBs (polychlorinated biphenyls). However, it is not designed to accurately determine extractable concentrations for volatile organics. The WET test is quite aggressive but it is not interchangeable with the TCLP test.

The WET involves mixing of the solid waste or material with citric acid extract (S/L of 1/10). The acid buffer solution is designed to simulate leaching characteristics which may occur in a non-hazardous solid waste landfill. The extraction process takes 48 hours. In some cases though it may be appropriate to use deionized water to accurately assess the leachability of contaminants in wastes without acid generation potential or with sufficient potential to neutralize all the acids formed in the waste (U.S. EPA, 1989a).

4.1.6 Shake Extraction of Solid Waste with Water or Neutral Leaching Procedure

The Shake Extraction of Solid Waste with Water or the Neutral Leaching Procedure was developed by the American Society for Testing and Materials (ASTM) to assess the leaching potential of solid waste (designated as ASTM D-3987-85). For this test the solid waste is mixed with an extractant, for example water, and the aqueous phase obtained is analyzed. The final pH of the extract reflects the interaction of the liquid extractant with the buffering capacity of the waste. However, this test has only been approved for certain inorganic compounds and is not applicable to organic substances and volatile organic compounds (www.epa.gov/industrialwaste).

4.2 Soil toxicity on biological life

Soil toxicity tests are generally designed to evaluate or detect the lethal or sublethal effects of various compounds on organisms in soil ecosystems and measure an endpoint or groups of endpoints such as mortality, reproductive capacity, growth rate, lesions and bioaccumulation, over a range of known concentrations of a compound. The results are then analyzed to determine the nature of the dose-response relationship. As shown in Figure 1, soil ecosystems are complex with great heterogeneity in physical, chemical and biological characteristics and are influenced by factors such as geology, topography, climate and anthropogenic activities (Torstensson 1997; Cal/EPA, 2009).

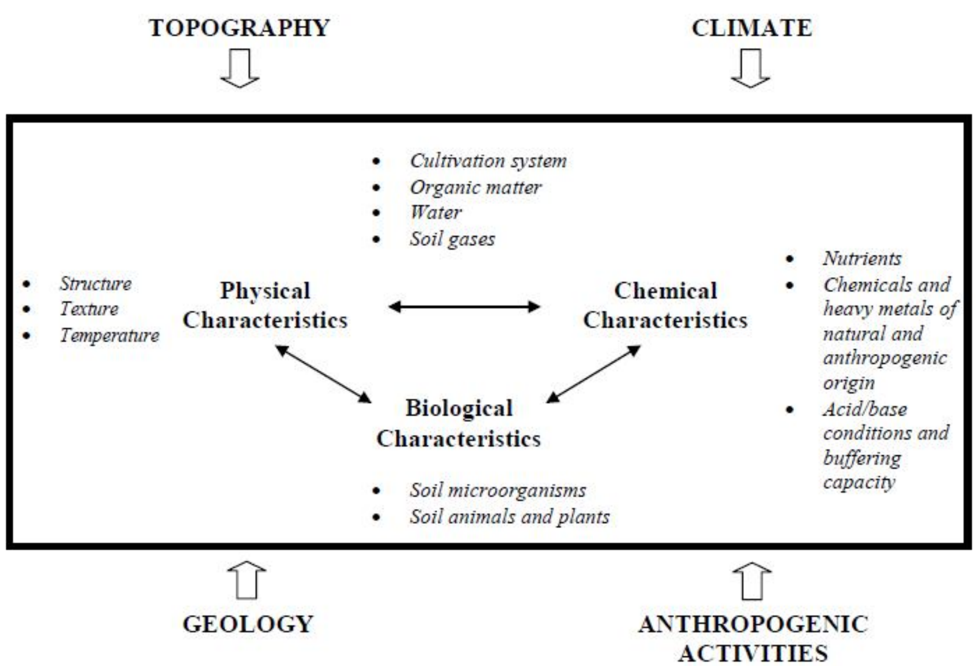

Figure 1. The complex structure of soil and its influences

4.2.1 Soil toxicity tests using microbial organisms

Soil toxicity tests using microbial organisms generally measure the functionality of the microbial community. This is in contrast to studies in which measured endpoints include individual organism lethality/survival or reproductive success or the bioaccumulation of test material. The toxicity to microbial communities is often measured in terms of a change in the community's ability to decompose organic matter and release plant nutrients. Microbial soil toxicity studies are commonly conducted by adding the test material to a soil core containing the naturally occurring microbial community to assess the effects of the chemicals on the ability of the community to maintain its functionality. When contaminated soils are the target of interest, microbial community function is compared against that of control soils. Inhibition of microbial community activity is thus a measure of adverse effect.

A list of standardized soil testing protocols and guidelines for microorganisms are shown in Table 4.

Table 4. Standardized soil testing protocols and guidelines for microorganisms (Cal/EPA, 2009)

Effect	Test guideline title	Test organism	Test duration	Endpoint
Acute	Soil micro-organisms: carbon transformation test	Soil microbes	28-100 days	Rate of respiration (mean carbon dioxide released in mg carbon dioxide/kg dry weight soil/h) or mean oxygen consumed in mg oxygen/dry weight soil/h)
Acute	Soil micro-organisms: nitrogen transformation test	Soil microbes	28-100 days	Nitrate production (mg nitrate/kg dry weight soil/day)
Acute	Luminescence bioassay	*Photo-bacterium phosphoreum* Strain NRRL B-11177	Approx. 1 hour	Quantitative reduction in light output of luminescent marine bacteria (i.e. IC20 or the calculated concentration of sample that would produce a 20% reduction in the light output of exposed bacteria over a specified time)
Acute	Soil microbial community toxicity test	Soil microbes	28 days	Ammonification and nitrification (measured as NH_3 and NO_3 concentration per gram of soil, respectively) and respiration (CO_2) eflux
Chronic	Soil-core microcosm test	Soil microbes	12 weeks or longer	Effect of chemicals on 1) growth and reproduction of either naturally occurring vegetation or crop(s) of interest, 2) nutrient uptake and cycling within the soil/plant system, 3) potential bioaccumulation (enrichment) of test material into plant tissue and 4) the potential for and rate of transport of the chemical through soil to ground water

4.2.2 Soil toxicity tests using plants

Soil toxicity tests using plants generally measure adverse effects on seeds or plants. Effects on individual plants are used to assess possible population or community level effects. For example, some species may be tolerant of a given toxicant, while others are highly sensitive. In natural ecosystems, changes in species diversity or in abundance may also influence the distribution and abundance of dependent wildlife species.

The assessment of phytotoxicity is one of the major criteria if using soil, sludge or composted biowaste as well as any kind of plant substrate (growing media) and soil improvers. Phytotoxicity is defined as a delay of seed germination, inhibition of plant growth or any adverse effect on plants caused by specific substances (phytotoxins) or growing conditions. Generally, there are two possible approaches to assess phytotoxicity: a) to grow plants directly in the test material or in diluted samples and b) to grow plants in hydroponic systems supplied with leachate. In some cases the use of closed systems is suggested to assess possible effects of volatile phytotoxins.

Phytotoxicity tests include hundreds of methods for the evaluation of toxic effects of chemicals on plants and vary depending on testing organism, test conditions and purpose. An overview of selected methods for the assessment of phytotoxicity on plants is discussed by Baumgarten and Spiegel (2004). Standardized soil testing protocols and guidelines for plants are shown in Table 5.

Table 5. Standardized soil testing protocols and guidelines for plants (Cal/EPA, 2009)

Effect	Test guideline title	Test organism	Life stage	Test duration	Endpoint
Acute or sub-chronic	-	Seedling	Early	14-28 days	Assessment of germination, seedling emergence, biomass (fresh or dry shoot weight, or shoot height) and visual detrimental effects (chlorosis, necrosis, wilting, mortality, abnormalities in development of leaf and stem)
Acute or sub-chronic	-	Seedling	Early	Until 65% of control seeds have germinated	Seed germination rate, root length
Acute or sub-chronic	-	Germinated seeds	Early	14 days	Mass and length of roots, shoots and entire plants
Acute or sub-chronic	-	Woody species (cutting or immature plant)	Early to maturing	Variable	Total plant weight, number of shoots or leaves, etc. Visual detrimental effects (same as above)
Chronic	*Brassica* life-cycle	Plant	Whole life-cycle	42 days	Wet and dry foliar and root weights, maximum foliar height, stem diameter, number and length of axillary stems, number of siliques, number and size of seeds

Depending on the scope of the method, several plants can be used for the phytotoxicity test (Table 6) varying in sensitivity to different test materials and toxins. At least, one monocotyledonous (category 1) and one dictyledonous plant (category 2) has to be used as test plants.

Various growing conditions (humidity, lighting, temperature) may be also considered to ensure optimum germination and growing conditions for the plants. The following conditions are recommended for the optimum growth of all selected species: a) temperature between 15 °C and 25 °C, b) lighting of 12-16 hours per day; 3000 lx minimum light intensity in the wavelength suitable for photosysnthesis (in a greenhouse additional lighting may be necessary when natural light is low, while the pots shall be shaded from direct sunlight), c) pH of soil between 5 and 7 (Baumgarten and Spiegel, 2004).

Phytotoxicity testing may be also used when performing ecological risk assessments to evaluate the efficacy of a selected remedial action, develop soil quality criteria or establish soil cleanup criteria.

Table 6. Test plants (in accordance with ISO 11269-2) (Baumgarten and Spiegel, 2004)

Category	Test plant	Scientific name
1	Barley (spring or winter)	*Hordeum vulgare L.*
1	Rye	*Secale cerale L.*
1	Ryegrass, perennial	*Lolium perenne L.*
1	Rice	*Oryza sativa L.*
1	Oat (common or winter)	*Avena sativa L.*
1	Wheat, soft	*Triticum aestivum L.*
1	Sorghum, common (or shattercane or durra, white or millet, great)	*Sorghum bicolor (L.) Moench*
1	Sweetcorn	*Zea mays L.*
2	Chinese cabbage	*Brassica campestris L. var. chinensis*
2	Cress, garden	*Lepidium sativum L.*
2	Mustard, white	*Sinapis alba*
2	Rape (summer or winter)	*Brassica napus (L.) ssp. napus*
2	Radish, wild	*Raphanus sativus L.*
2	Turnip, wild	*Brassica rapa ssp. rapa(DC.) Metzg.*
2	Bird's foot clover, Fenugreek	*Trifolium ornithopodioides L.*
2	Lettuce	*Lactuca sativa L.*
2	Tomato	*Lycopersicon esculentum Miller*
2	Bean	*Phaseolus aureus Roxb.*

4.2.3 Soil toxicity tests using invertebrates

Soil toxicity tests using invertebrate species are generally used to characterize lethality, reproductive success or bioaccumulation, in contrast to the functionality studies where the study endpoint is the function of the test species within the ecosystem. Invertebrate soil toxicity studies are commonly conducted by adding a known number of test organisms to the test material (either soils collected from the field or spiked artificial soils) to assess the effects of contaminants on the test organisms. Results are measured as the number of organisms surviving (or dead) or observations of sub-lethal behaviors and/or reproductive success (number of cocoons or juveniles).

A list of standardized soil testing protocols and guidelines for invertebrates is seen in Table 7.

Table 7. Standardized soil testing protocols and guidelines for invertebrates (earthworms) (Cal/EPA, 2009)

Effect	Test guideline title	Test organism	Life stage	Test duration	Endpoint
Acute	Earthworm, acute toxicity tests	*Eisenia fetida*	Adult	Filter paper test: 48-72 hours, artificial soil test: 14 days	Mortality and other noted abnormal behaviors
Sub-chronic	Earthworm, sub-chronic toxicity tests	*Eisenia fetida*	Adult	28 days	Mortality and other noted abnormal behaviors, pathological conditions or weight loss
Sub-chronic	Soil toxicity or bioaccumu-lation tests with the Lumbricid Earthworm *Eisenia fetida*	*Eisenia fetida*	Adult	14-28 days	Endpoints are dependent on purpose of the test and study design but may include animal weight, lethality, sublethal behaviors, pathological changes (segmental constriction, lesions, stiffness, etc.), reproduction, tissue accumulation, etc. Other endpoint analysis may include kinetic studies with estimate uptake, depuration rates, and time to steady state, lipid normalization and normalizing soil concentrations of non-ionic organics to total organic carbon. Reproductive endpoints might include number and growth of young worms, rate of clitellum development, number of cocoons produced, cocoon mass, number of hatchlings per cocoon and biomass of hatchlings.
Chronic	Earthworm Reproduction Test (*Eisenia fetida/ andrei*)	*Eisenia fetida/andrei*	Adult	8 weeks observation. Adult earthworms are removed after the fourth week	Adult mortality and other signs of toxicity, reproductive success as measured by number of juveniles produced.

4.2.4 Soil toxicity tests using vertebrates

Standardized vertebrate toxicity tests are generally single species laboratory tests. Measured endpoints include individual organism lethality/survival, reproductive success, behavioral abnormalities or the bioaccumulation of test material in target tissues or whole bodies. Standardized toxicity tests for vertebrate species have not been developed with the single view of assessing the effects of anticipated or known soil contaminants on likely soil inhabiting vertebrate species. Standardized tests are rarely being conducted using the animals and all the exposure routes of concern, but have been designed to assess a variety of chemicals through one route of exposure at a time and species such as mice, rat, Coturnix quail, chicken, dog and rabbit species, are tested under laboratory conditions (Cal/EPA, 2009).

Vertebrate toxicity tests generally fall within one or more “classical” approaches to toxicity determination, including:

- Acute Toxicity Test (Oral lethal dose 50% LD_{50}, dermal LD_{50}, inhalation lethal concentration 50% LC_{50}, dietary LC_{50})
- Subchronic and Chronic Tests (reproductive, fertility, prenatal development, carcinogenicity, genetic toxicity, neurotoxicity and other specialized tests)

4.3 Water toxicity

A compound may be potentially hazardous to the aquatic environment if it has one or more of the following three properties (UNECE, 2003):

1) Aquatic toxicity: the hazard of a substance to living organisms based on toxicity tests to aquatic animals and plants
2) Degradability: the persistence of the substance in the environment based on molecular structure or analytical testing
3) Bioaccumulation/bioconcentration: the accumulation of a substance in living organisms (from water sources for bioconcentration), which may or may not lead to a toxic effect; based on calculations or bioconcentration factor (BCF) studies using fish

The adverse effects or toxicity in surface waters can be detected and addressed through Whole Effluent Toxicity (WET) testing developed by the U.S. EPA. Toxicity measured by WET testing can be caused by a number of factors that act independently or jointly, including:

- Chemical factors (inorganic chemicals such as ammonia, chlorine and heavy metals; organic chemicals such as dioxins, polychlorinated biphenyls (PCBs) and surfactants; pesticides such a chlorpyrifos, diazinon and heptachlor)
- Physical factors (dissolved and suspended solids; temperature)
- Biological factors (bacteria; fungi; parasitic invertebrates)

Toxicity can be experimentally determined by exposing sensitive organisms to effluents and assessing responses such as survival. Test organisms include vertebrates, invertebrates and plants. Examples of organisms frequently used in WET testing are:

a) freshwater organisms such as fish eg. fathead minnow (*Pimephales promelas*); invertebrates eg. daphnids (*Ceriodaphnia dubia*, *Daphnia magna* and *D. pulex*); algae eg. green alga (*Raphidocelus subcapitata*, formerly *Selenastrum capricornutum)* and

b) saltwater organisms such as fish eg. sheepshead minnow (*Cyprinodon variegates*) and inland silverside (*Menidia beryllina*); invertebrates eg. mysid (*Americamysis bahia*, formerly *Mysidopsis bahia*) and sea urchin (*Arbacia punctulata*); algae eg. red alga (*Champia parvula*).

WET tests can be performed under a variety of dilution conditions and exposure periods. The duration of the test may range from periods as short as 40 minutes up to 7 days depending on the organisms used and whether acute or chronic effects are of interest. Acute tests are conducted for a relatively short period and usually focus on how well an organism survives, while chronic tests are conducted for longer periods relative to certain organisms' life cycle or during a very sensitive life stage to evaluate survival, growth and/or reproduction (SETAC, 2004).

Once the toxicity of a sample is estimated, the result is then compared to the respective water quality standards to determine if sample quality is acceptable. Drinking water standards and Netherlands thresholds for groundwater for selected parameters are shown in Table 8.

5. The case of OMW toxicity

Most of the technologies developed for AW treatment focus on the reduction of AW toxicity which is mainly related to the degradation of recalcitrant compounds such as phenols or the decrease of COD, emphasizing also the role of various ecotoxicological tests. For instance, different groups such as bacteria, crustacean, plants and various tests have been used to assess the toxicity of OMW, as shown in Table 9; ecotoxicological data for the endpoints evaluated are also shown. It is mentioned that toxicity of tested OMW depends on specific conditions for each study (Justino et al., 2012).

It has to be mentioned though that no threshold values exist at EU level to assess phytotoxicity of various contaminants, for example phenols, contained in OMW (Mekki et al., 2008; Justino et al., 2012). Also, no ecotoxicological data are available for the evaluation of toxicity of OMW on edaphic invertebrate species after soil irrigation with OMW as well as on various endpoints in standard crop species (growth above ground or growth of roots). Therefore, the application of a battery of biomarkers to assess the cytotoxic and genotoxic effects of OMW on aquatic invertebrate species (*Mytillus galloprovinciallis*) and aquatic animal species, is strongly recommended (Danellakis et al., 2011).

Due to the lack of pertinent legislation and by taking into account the variability of soils and the quality of OMW produced in various countries, it would have been very helpful if quantitative, reliable and widely acceptable indicators were developed to define appropriate rates for waste application on soils and assess long term beneficial or adverse effects on ecosystems (Bouma and Imeson, 2000; Stocking and Murnaghan, 2001).

Regarding management of wastes, no integrated specific EU legislation exists and thus each country issues different guidelines. For instance, liquid wastes from olive oil production fall under the Urban Waste Water Treatment Directive (91/271/EEC) involving collection, treatment and discharge. In Italy application of OMW on soil is only allowed under certain circumstances (Altieri and Esposito, 2010), while in Greece application of untreated OMW on soils is not allowed (Greek Laws 1650/86 and 3010/2002). Indicative waste disposal limits regarding water receivers in Greece are shown in Table 10.

Directive 1999/31/EC of 26 April 1999 related to the landfilling of waste provides license applications and technical requirements for the design, operation, monitoring, closure and post closure care for landfills in order to prevent or minimize negative effects on the environment (pollution of surface- and groundwater, soil and air) as well as on human health. The different categories of waste (municipal, hazardous, non-hazardous and inert waste) as well as the types of landfills (landfill for hazardous waste, non-hazardous and inert waste) are defined and categorized.

The Waste Framework Directive (2008/98/EC) includes rules on hazardous waste and waste oils and requires Member States to recycle at least half of their household and general waste by 2020. According to the hierarchy, waste should be dealt with first by prevention, then re-use, recycling, recovery and finally disposal. During recovery, waste is either converted into usable forms or is incinerated so that energy is recovered. Disposal, meaning landfilling in most cases, can only be done once the previous four steps have been exhausted.

Table 8. Drinking water standards and Netherlands thresholds for groundwater for selected parameters

Parameter	Drinking water standards	Netherlands thresholds: Target Value groundater[1] <10 m	Netherlands thresholds: Target Value groundater[1] >10 m	Netherlands thresholds: Intervention Value groundwater[2]
pH	6.5-8.5 (FPTC, 2010)	-		-
Electrical conductivity, µS/cm	~2500 (20 ^{o}C) (98/83/EC)	-		-
$Hardness_{tot}$, mg/L $CaCO_3$	Acceptable 80-100 mg/L, tolerable >200 mg/L but unacceptable >500 mg/L (FPTC, 2010)	-		-
Phenols, µg/L	No health-based guideline value (suggested safe levels <0.5 µg/L) (CMD Y2/2600/2001)	0.2		2000
NO_3^-, mg/L	<45 (FPS, 1999; FPTC, 2010)	-		-
SO_4^{2-}, mg/L	≤250 (98/83/EC; CMD Y2/2600/2001)	-		-
PO_4^{3-}, mg/L	No health-based guideline value (suggested safe levels <5) (WHO, 2008)	-		-
Cl, mg/L	<250 (98/83/EC; FPS, 1999)	-		-
K, mg/L	<12 (98/83/EC)	-		-
Na, mg/L	<200 (98/83/EC)	-		-
Fe, mg/L	<0.2 (98/83/EC)	-		-
NH_3-N, mg/L	<3 (WHO, 2008)	-		-
Cu, mg/L	<2 (98/83/EC)	0.015	0.0013	0.075
Zn, mg/L	≤5 (FPTC, 2010)	0.065	0.0024	0.8
Mn, µg/L	<50 (98/83/EC; FPTC, 2010)	-		-
Ni, µg/L	<70 (WHO, 2008), <20 (98/83/EC)	15	2.1	75
Mo, µg/L	<70 (WHO, 2008)	5	0.7	300
Cr, µg/L	<50 (98/83/EC; FPTC, 2010)	1*	2.4*	30*
As, µg/L	<10 (98/83/EC; FPTC, 2010)	10	7.2	60
Cd, µg/L	<5 (98/83/EC)	0.4	0.06	6
Co, µg/L	-	20	0.6	100
Pb, µg/L	<10 (98/83/EC)	15	1.7	75

[1] Negligible risk to ecosystems, 1% of the Maximal Permissible Risk level for ecosystems (MPReco)

[2] Risk for ecosystems and processes, as well as human risk (related mainly to pore water concentration, rather than the total soil concentration)

- No thresholds exist

* Based on Cr^{+3} only

Table 9. Ecotoxicological data for the assessment of the toxicity of OMW on aquatic and terrestrial species (Justino et al., 2012)

Taxonomic group	Test organism	Endpoint	Toxicity of raw OMW according to other studies
Bacteria	*Vibrio fischeri*	Luminescence inhibition	100% or $0.2\% < EC_{50} < 1.2\%$ for OMW produced from discontinuous traditional process; LC_{50} 3.5% for OMW produced from 3-phase system; 96% for 24 times diluted OMW
	Bacillus megaterium, Escherichia coli and Pseudomonas fluorescens	Growth inhibition	73 to 100% for 50% diluted OMW
	Non defined consortium	N.I.	EC_{50COD} ~ 5,500 mg/L
Algae	*Pseudokirchneriella subcapitata*	Growth inhibition	100% for diluted OMW (1:160)
Rotifers	*Brachionus calyciflorus*	Mortality	N.I.
Crustaceans	*Daphnia magna*	Growth inhibition	$1.1\% < EC_{50} < 6.8\%$
	Daphnia longispina	Growth inhibition	$5.3\% < EC_{50} < 9.9\%$ for OMW produced from 3-phase system
	Gammarus pulex	Mortality	LC_{50} 3% for OMW produced from 3-phase system
	Thamnocephalus platyurus	Mortality	$0.7\% < EC_{50} < 12.5\%$ for OMW produced from discontinuous traditional process
Arthropods	*Heterocypris incongruens*	Mortality	LC_{50} 3.7% for OMW produced from 3-phase system
	Hydropsyche peristerica	Mortality	LC_{50} 3.8% for OMW produced from 3-phase system
Plants	Maize, wheat, chickpea, tomato, barley, English cress, durum wheat, radish, cucumber and lettuce	Inhibition of seed germination, growth, ramifications and leaf extension rates	N.I.
	Vicia faba root tips	Micronucleus frequency	N.I.

LC_{50} (lethal concentration 50): measure of the toxicity of the surrounding medium that will kill half of the sample population of a specific test-animal in a specified period through exposure via inhalation (respiration).

EC_{50} (half maximal effective concentration): concentration of a toxicant which induces a response halfway between the baseline and maximum after some specified exposure time.

N.I.: not identified

Table 10. Indicative waste disposal limits regarding water receivers in Greece

Parameter	Perfecture of Chalkidiki (CMD 573 v2, 24/9/85)	Saronikos gulf (CMD 1132 v2, 21/12/79)	Sewer in Keratsini Piraeus (CMD 582 v2, 2/7/79)	Streams in Keratsini Piraeus (CMD 582 v2, 2/7/79)
Al, mg/L	5	5	10	1
As, mg/L	0.5	0.5	0.5	0.1
Ba, mg/L	10	20	20	2
B, mg/L	1	2	10	2
Br, mg/L	-	-	10	1
Cd, mg/L	0.02	0.1	0.5	0.05
Cr^{+3}, mg/L	2	2	2	1
Cr^{+6}, mg/L	0.2	0.2	0.5	0.2
Cu, mg/L	0.5	1.5	1	0.2
Fe_{tot}, mg/L	15	2	15	2
Pb, mg/L	0.1	0.1	5	0.5
Mn, mg/L	2	2	10	1
Hg, mg/L	0.005	0.005	0.01	0.01
Ni, mg/L	2	2	10	0.5
Zn, mg/L	2	1	20	0.5
P, mg/L	10	10	10	0.2
Suspended solids, mg/L	40	40	500	50
F, mg/L	6	6	20	2
Cl_{free}, mg/L	1	0.7	5	0.4
NO_3, mg/L	100	20	20	4
NO_2, mg/L	5	0.6	4	1
NH_4, mg/L	20	15	25	10
$MeSO_3$, mg/L	2	1	1	0.2
MeS, mg/L	2	2	-	-
$MeSO_4$, mg/L	-	-	1500	1000
H_2S, mg/L	-	-	1	0.1
pH	6 - 8.5	6 - 9	6 - 9	6 - 9
Dissolved oxygen, mg/L	-	-	-	3
BOD_5, mg/L	40	40	500	40
COD (120min method), mg/L	150	150	1000	120

6. Compost as soil improver

The application of treated organic wastes (compost) on soil improves soil fertility, increases soil organic matter and nutrients content, improves physical properties of soil such as aggregate stability, enhances crop production and contributes to minimization of risk for soil, water and ecosystems. Compost can replace fertilizer in many applications such as in commercial greenhouse production, farms and land remediation contributing also to fertilizer cost reduction.

Fertilizer is defined as any substance containing one or more recognized plant nutrients to improve plant growth. A fertilizer either (a) contains important quantities of no more than one of the primary plant nutrients (N, P, K) or (b) has 85% or more of its plant nutrient content present in the form of a single chemical compound or (c) derives from a plant or animal residue or by-product or natural material deposit which has been processed in such a way that its content of plant nutrients has not been materially changed except by purification and concentration (AAPFCO, 1997).

Table 11 shows the average, high and maximum application rates of fertilizers (represented as 50^{th}, 85^{th} and 95^{th} percentiles, respectively) in kg/ha used in calculation of metal addition to soil to improve the growth of crops, vegetables and fruits. It is assumed that all fertilizer products are applied annually. It is mentioned that variations in the percentage of the fertilizer's active ingredient are seen; for example, the percentage of P_2O_5 in the phosphate fertilizers ranges from 15 to 53 %.

Compost is produced either aerobically or anaerobically. Aerobic composting is the most efficient form of decomposition and a short time is required; optimal conditions for selected parameters are seen in Table 12 (http://www.cias.wisc.edu/wp-content/uploads/2008/07/artofcompost.pdf).

The main factors to be taken into consideration for a successful composting include the chemical composition of the waste used, the size and shape of the feedstocks (porosity of the pile) and the population of organisms involved. Also, pH and electrical conductivity (EC) should be considered due to their effect on physicochemical and microbiological reactions taking place in soil. It is mentioned that during the first days of composting, pH shows significant fluctuation due to ammonium release through volatilization and nitrification taking place in the bio-oxidation phase of degradation when organic acids are formed and polysaccharides are decomposed. From an agricultural point of view, pH values should range between 6.5 and 8 (as shown in Table 12). Soil pH is also a reliable indicator of Al toxicity, which is in general the most limiting factor of crop production in acid soils (pH <5.5). In alkaline soils, the solubility of Al and Mn compounds is limited and thus toxicity to plants is substantially reduced (Norton et al., 1999). Soils with pH>7 and high content in $CaCO_3$ have the ability to buffer waste acidity and thus, no significant effect on soil pH is anticipated (Banegas et al., 2007; Zaharaki and Komnitsas, 2012).

Compost EC plays a significant role in plant growth and seed germination. When EC is higher than 8 mS/cm, negative effects on soil microbial populations as well as on organic matter biotransformation have been observed (García and Hernández 1996; Santamaría-Romero and Ferrera, 2001; Banegas et al., 2007).

Various other parameters to be determined include colour, temperature, moisture, organic matter, C/N ratio, humification index, cation exchange capacity, humic/fulvic acids ratio, COD, germination index as well as N, P, K, Ca, Mg, Fe, Mn, Zn, Cu, Ni, Pb, Cd, and Cr content. It is mentioned that organic matter is a critical soil property which is involved in all soil functions and affects physical, chemical and biological processes in soils. Also, high water holding capacity may contribute to the adsorption of micronutrients after waste disposal and increase soil fertility as well as reduce its toxicity (Roca-Pérez et al., 2009).

Nitrogen is the most important micro-element required for plant development. However, excess of nitrogen, caused by fertilization or waste disposal, may result in water eutrophication or cause health problems to humans.

Table 11. Application rates (kg/ha) of fertilizers per unit active ingredient (U.S. EPA 1999; U.S. EPA and CEA, 1999)

Fertilizer type (active ingredient)	50th percentile (average), kg/ha	85th percentile (high), kg/ha	95th percentile (maximum), kg/ha
Phosphate (P_2O_5)	94	194	282
NPK applied for phosphorus (P)	94	194	282
NPK applied for nitrogen (N)	139	231	464
Potash (K_2O)	115	198	599
Sulfur (nutrient)	22	45	67
Sulfur (pH)	897	2242	2802
Lime[1] ($CaCO_3$)	4483	897	16812
Gypsum	2242	4483	8967
Iron	11	22	34
Boron	2	3	4
Manganese	4	11	20
Zinc	6	11	22
Micronutrient mixes	8	NA	NA

[1] Lime is typically applied every 2-3 years but the application rates have been normalized based on $CaCO_3$ content to provide annual application rates. Additionally, the distribution includes a sample which contains only 7.6% $CaCO_3$. Up to 10 times as much of this product must be used to achieve the same liming effect as most other products.

NA = Not available

Table 12. Optimal conditions for selected parameters, for the aerobic production of compost

Parameter	Acceptable values	Optimum values
C:N ratio	20:1 to 40:1	25:1 to 35:1
Moisture content	40-65% w/w	45-60% w/w
Available oxygen concentration	>5%	>10%
pH	5.5-9	6.5-8
Temperature	43-66 ^oC	54-60 ^oC

Phosphorus in soils exists in inorganic and organic form and is also considered as essential element for plant growth. However, P can be rapidly fixed in forms unavailable to plants, depending on soil pH and type as well as on Al, Fe, and Ca content. It is very important though to establish the relation between total and plant available P and thus specify fertilization needs in order to maximize yield (Watson and Mullen, 2007).

Regarding potassium, it is mentioned that only a small percentage, not exceeding 1%, of the total K in soils is exchangeable. Exchangeable K, which is considered the primary source of K for plant uptake, ranges from <100 to 2,000 mg/kg, while total K values are normally in the order of 1 to 2%. In highly weathered soils or soils where the parent material contains only some K-bearing minerals, the exchangeable K can be depleted by plant removal and is replenished only by fertilizer or waste application or return of K from plant residues.

7. Heavy metal loading limits for soil improvers application

Soil improvers such as compost may be produced by co-utilization of various treated or untreated AW considering also the use of sewage sludge which improves soil properties due to its nitrogen, phosphorous and trace elements content. However, sewage sludge should be treated properly to eliminate pathogens and phytotoxicity.

According to Directive 86/278/EEC of June 12, 1986 on the "protection of the environment and in particular of the soil, when sewage sludge is used in agriculture" aiming to prevent adverse effects on soil, vegetation, animals and people, limit values for concentrations of heavy metals in soil, sludge as well as for the maximum annual quantities of heavy metals which may be introduced into the soil, have been defined (Table 13). This Directive has been amended by Directive 91/692/EEC of 23 December 1991 and Regulation (EC) No 1882/2003 of the European Parliament and of the Council of 29 September 2003.

Table 13. Limit values for concentrations of heavy metals in soil, in sludge for agricultural use as well as for the maximum annual quantities of heavy metals which may be added in agricultural land

Parameter	Limit values for concentrations of heavy metals in soil (mg/kg of dry matter of soil with a pH of 6-7)	Limit values for concentrations of heavy metals in sludge for agricultural use (mg/kg of dry matter)	Limit values for the maximum annual quantities of heavy metals which may added in agricultural land, based on a 10-year average (kg/ha/y)
Cd	1-3	20-40	0.15
Cu	50-140	1000-1750	12
Ni	30-75	300-400	3
Pb	50-300	750-1200	15
Zn	150-300	2500-4000	30
Hg	1-1.5	16-25	0.1
Cr	not defined	not defined	not defined

Table 14 provides a comparison between annual metal loading limits according to the Canadian standards and sewage sludge pollutant loading rates on land, provided by the U.S. Environmental Protection Agency (EPA). It is important to note that the Canadian standards are not based on a quantitative assessment of risk but instead reflect the best professional judgment of a number of experts on metals behavior in soils and plants. The standards consist of both maximum annual loadings and maximum acceptable cumulative additions of metals contained in fertilizers.

Table 14. Comparison between annual metal loading limits according to the Canadian standards and sewage sludge pollutant loading rates on land, provided by the U.S. EPA (U.S. EPA and CEA, 1999)

Metal	Canadian annual loading rate (kg/ha/y)[a]	Sewage sludge annual pollutant loading rates (kg/ha/y)[b]
As	0.33	2
Cd	0.09	1.9
Cr	NA	NA
Cu	NA	75
Pb	2	15
Hg	0.02	0.85
Ni	0.8	21
Se	NA	5
Zn	8	140

[a] Fertilizer products are assumed to be applied annually at a rate of 4,400 kg/ha for 45 years. Limits are for products containing 5% N or less. Acceptable concentrations increase proportionally with total N concentrations above %.

[b] Sewage sludge is assumed to be applied annually by home gardeners for 20 years or semiannually for 40 years.

The California assessment provides also risk-based concentrations (RBCs) for lead, arsenic and cadmium in fertilizers and micronutrients. California's RBCs may be converted to annual loading limits for different types of fertilizers or micronutrients by using (1) the appropriate RBCs combined with (2) corresponding assumptions regarding fertilizer composition (i.e. % phosphorus) and/or micronutrient composition (i.e. % zinc) and (3) corresponding assumptions regarding expected annual application rates. Table 15 shows calculated annual application rates for each metal and crop type. These application rates are calculated using the annual nutrient application rates used by California to generate RBCs and the most conservative RBCs derived for each compound (U.S. EPA, 1999).

8. Assessment of risk for humans due to fertilizer application

This risk assessment is designed to estimate the increase in lifetime risk to human (mainly farmers and their children) who are exposed to various recalcitrant compounds such as metals contained in treated or untreated AW. Both direct and indirect exposure to contaminants through ingestion of vegetables produced on fertilized soil or animals fed in these areas, should be taken into consideration. Therefore the concentrations for each metal in soils, surface water, plant tissue (fruits, vegetables, grains and forage) and animal tissue (fish and beef and dairy products) should be measured (U.S. EPA and CEA, 1999).

The following parameters are to be taken into consideration:

- characterization of fertilizers produced i.e. composition, patterns of use and application rates
- geographical location of the implementation area i.e. agricultural land use, climate data, soil data, farm size, crop types and plant uptake factors
- description of receptors i.e. body weight and inhalation rate for humans, as well as exposure pathways and duration

Table 15. Calculated application rates for California risk-based concentrations

Metal	Product	Application rate (kg/ha/y)
	Vegetables	
Pb	P_2O_5	0.654
	Micronutrients	0.494
As	P_2O_5	0.128
	Micronutrients	0.104
Cd	P_2O_5	0.108
	Micronutrients	0.09
	Roots	
Pb	P_2O_5	0.720
	Micronutrients	0.494
As	P_2O_5	0.141
	Micronutrients	0.104
Cd	P_2O_5	0.119
	Micronutrients	0.09
	Grains	
Pb	P_2O_5	0.407
	Micronutrients	0.494
As	P_2O_5	0.08
	Micronutrients	0.104
Cd	P_2O_5	0.067
	Micronutrients	0.09

The general framework of the ecological risk assessment is shown in Table 16 and consists of three major phases: 1) problem identification, 2) analysis and 3) risk characterization (U.S. EPA 1992).

The contaminant fate and transport models are used to determine contaminant concentrations in the soil, to estimate risk from the air pathway or from surface water as well as to estimate exposure point concentrations in the food chain. The relevant exposure routes for humans are:

- Direct ingestion of the compost (fertilizer) during application
- Ingestion of soil amended with compost
- Inhalation of particles and vapors in the air during and after compost application
- Ingestion of plant, vegetables and fruits produced on amended soil as well as animals fed in this soils
- Ingestion of fish from streams located adjacent to amended fields

The dose-response assessment determines the most sensitive health effects associated with a compound and attempts to express the relationship between dose and effect in quantitative terms, known as toxicity values or health benchmarks. Generally, health benchmarks are developed by EPA in four types: reference doses (RfDs), reference concentrations (RfCs), cancer slope factors (CSFs) and unit risk factors (URFs).

Table 16. Critical phases of the ecological risk assessment

Phase I	Problem identification	- Identify stressor characteristics such as type, intensity, duration, frequency, timing, scale - Identify the ecosystem potentially at risk - Evaluate existing data of ecological effects - Select appropriate endpoints, considering ecological relevance, policy goals and societal values, susceptibility to the stressor - Develop a conceptual model, working hypothesis regarding how the stressor might affect the ecological components of the ecosystem
Phase II	Analysis	*Characterization of exposure*: - Characterize the stressor in terms of distribution or pattern of change - Characterize the ecosystem - Analyze the potential exposure - Develop an exposure profile *Characterization of ecological effects*: - Evaluate the relevant effects data - Analyze the ecological response in terms of stressor – response determinations or extrapolations and causal evidence evaluation - Develop a stressor-response profile
Phase III	Risk characterization	- Estimate the risk - Integrate the stressor-response and exposure profiles - Identify uncertainty in the analyses - Describe the risk - Summarize the risk assessment - Interpret the ecological significance

RfDs and RfCs, used to evaluate non-cancer effects for ingestion and inhalation exposures, respectively, are defined as an estimate of a daily exposure level for the human population, including sensitive subpopulations that is likely to be without an appreciable risk of deleterious effects during a lifetime (U.S. EPA, 1989b). RfDs are expressed in mg of chemical intake per kg body weight per day (mg/kg/d) and RfCs are expressed as mg of chemical per m^3 of air (mg/m^3). RfCs may be converted into inhalation RfDs in mg/kg/d by multiplying by the inhalation rate and dividing by the body weight.

CSFs and URFs may be derived from a number of statistically and/or biologically based models. CSFs and URFs are used to evaluate cancer effects for ingestion and inhalation exposures, respectively. However, they do not represent “safe” exposure levels; rather they are expressed as an upperbound slope factor that relates levels of exposure with a probability of effect or risk. The CSF is expressed in units of $(mg/kg/d)^{-1}$ and the URF for inhalation exposures is expressed in units of $(\mu g/m^3)^{-1}$.

The health benchmark values used in risk analysis for metals were developed by U.S. EPA and are presented in Table 17.

Table17. Health benchmark values for metals in fertilizers (U.S. EPA and CEA, 1999)

Metal	RfD (mg/kg/d)	RfC (mg/m^3)	CSF oral $(mg/kg/d)^{-1}$	Inhalation URF $(\mu g/m^3)^{-1}$
As	0.0003	NA	1.5	0.0043
Cd	0.001	NA	NA	0.0018
Cr	0.003	0.0001	NA	0.012
Cu	NA	NA	NA	NA
Pb	NA	NA	NA	NA
Hg (Elemental divalent methyl mercury)	0.0001	0.0003	NA	NA
Ni	0.02	NA	NA	0.00024
V	0.007	NA	NA	NA
Zn	0.3	NA	NA	NA

NA = Not available

The estimated exposure point concentration in soil, plants and animal products should be combined with toxicity benchmarks and exposure factors such as exposure duration and ingestion rates to estimate human health risk (U.S. EPA and CEA, 1999).

The risk for all ingestion pathways for a single constituent is assumed to be additive. Ingestion exposures are assumed to occur in the same time for the same individuals and the same health benchmark is applicable to all ingestion exposures. For constituents with non-cancerous endpoints, inhalation exposure is additive to ingestion exposure only if the same human health benchmark endpoint is applicable to both pathways.

Similar additions of risk may be considered for different metals in the same product. For metals, however, only arsenic is considered as carcinogen by the oral route. All other metals are not carcinogenic by the oral route and do not have common health benchmark endpoints; thus, ingestion exposures to different metal constituents in a single product are not considered additive. No other metal exposures are considered additive because there are no common target organs for the non-cancerous human health benchmarks for these metals (U.S. EPA and CEA, 1999).

9. Conclusion

In the present deliverable, the potential adverse effects of the disposal of the most important AW produced in the Med region, including olive oil mill wastes, wine, swine and other animal waste, rice straw and various other AW, on soils and water bodies are discussed. AW have a high contamination potential which is usually related to their high organic load and rather low pH, affecting thus living organisms, soils, water bodies and plants.

Toxicity is a very significant parameter for the characterization of AW and it should be taken into account before and after treatment to a) select the most appropriate treatment technologies which should reduce the toxicity of treated AW to acceptable levels, b) define the use of the final products and c) define the optimum management strategy of the secondary wastes produced in order to eliminate adverse impacts on humans and environment. Numerous toxicity tests for wastes, soil and water are used to assess the concentration of the contaminants in leachates and compare with thresholds as well as to evaluate the hazard of the contaminants to particular test organisms.

Composting of AW is the most commonly used management option and the final product can be used as soil improver to enhance crop production and minimize risk for soil, water and ecosystems.

It is mentioned that regarding management of AW, no integrated specific EU legislation exists and thus each country issues different guidelines. However, pre-treatment of AW, careful application on soils, use of standardized procedures to evaluate toxicity and determination of the fate of contaminants in soil and water will maximize sustainability in agriculture and minimize impacts on ecosystems.

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