UNIVERSIDAD DE MURCIA

ESCUELA INTERNACIONAL DE DOCTORADO

Evaluation of the Potential as Biofuels of Species Developed in Contaminated Soils

Evaluación del Potencial como Biocombustibles de Especies Desarrolladas en Suelos Contaminados

Donatella Grippi
2020

CSIC
Consejo Superior de Investigaciones Científicas

Centro de Edafología y Biología
Aplicada del Segura - CSIC

Universidad de Murcia
Escuela Internacional de Doctorado
Programa de Doctorado en
Biodiversidad y Gestión Ambiental

TESIS DOCTORAL

EVALUATION OF THE POTENTIAL AS BIOFUELS OF SPECIES DEVELOPED IN CONTAMINATED SOILS

EVALUACIÓN DEL POTENCIAL COMO BIOCOMBUSTIBLES DE ESPECIES DESARROLLADAS EN SUELOS CONTAMINADOS

Donatella Grippi
2020

Agradecimientos

Un agradecimiento especial para la Dra. María del Pilar Bernal Calderón y el Dr. Rafael Clemente Carrillo por su disponibilidad, paciencia y cordialidad, por haberme seguido y acompañado en esta maravillosa experiencia que me hizo retroceder en el tiempo, a los maravillosos años de la Universidad y me hizo recordar cuánto el mundo de la investigación científica es maravilloso y fascinante.

Además, pude combinar la investigación científica con la posibilidad de descubrir las bellezas de España.

Gracias a la Universidad de Murcia y al CEBAS CSIC por darme la oportunidad de realizar este trabajo de investigación y a la asociación Mnemosine que gestionó las relaciones.

Gracias también a mi familia que estuvo a mi lado y que me aguantó y me apoyó en los momentos de desánimo que a veces ocurrían.

INDEX

CHAPTER 2. THE USE OF ENERGY CROPS FOR THE RECOVERY OF TRACE ELEMENTS CONTAMINATED SOILS 95

1. RESUMEN

EVALUACIÓN DEL POTENCIAL COMO BIOCOMBUSTIBLES DE ESPECIES DESARROLLADAS EN SUELOS CONTAMINADOS

1. Resumen

La demanda mundial de producción de energía primaria aumenta cada vez más, con un crecimiento esperado del 1,4 % anual hasta el 2035 (Pimentel et al., 2017). También en el ámbito del transporte se espera que en 2030 el uso de la energía sea aproximadamente un 50 % superior al ocurrido en la década de los 90. Para hacer frente a la creciente demanda de producción de energía y, al mismo tiempo, abordar los problemas de protección del medio ambiente, la Unión Europea ha fijado dentro de la "Estrategia Europa 2020" el objetivo de aumentar la proporción de energías renovables al 20 %. Todo ello ha llevado a la necesidad de intervenir con medidas apropiadas para alcanzar objetivos económicos, sociales y medioambientales, como por ejemplo la reducción del consumo de combustible y electricidad, la contención de las emisiones a la atmósfera de gases de efecto invernadero (dióxido de carbono, metano, óxido nitroso y varios hidrocarburos), la mejora de la calidad ambiental en los centros urbanos, etc. La búsqueda de medidas efectivas para la mejora del medio ambiente se dirige hacia la sustitución progresiva de los combustibles fósiles tradicionales por los de origen biológico: materiales y residuos de origen agrícola y ganadero como residuos vegetales y estiércoles de animales, también subproductos de la industria agroalimentaria, y residuos urbanos.

Mediante la fitoestabilización, el establecimiento de una cubierta vegetal autóctona y autosuficiente sobre suelos contaminados podría reducir el riesgo de una transferencia

incontrolada de contaminantes al medio ambiente, promoviendo la remediación de los suelos contaminados y al mismo tiempo, estos procesos pueden proporcionar biomasa vegetal útil para la producción de (bio)energía. Esto puede considerarse una opción adecuada y respetuosa con el medio ambiente para lograr la cuota de energía renovable deseada.

El objetivo de esta Tesis fue proporcionar información relevante, actualizada y útil sobre el potencial de producción de bioenergía de las especies vegetales que se utilizan para la remediación de suelos mediante la fitoestabilización de suelos contaminados con elementos traza. Los objetivos parciales fueron:

- Revisar las principales técnicas de producción de energía;

- Determinar las características de las especies vegetales utilizadas para los diferentes bio-combustibles;

- Identificar especies vegetales nativas con características compatibles con la producción de bioenergía que, al mismo tiempo, puedan tolerar altos niveles de metales pesados y metaloides en los suelos donde se cultivan;

- Revisar el potencial de las especies vegetales que crecen espontáneamente en sitios contaminados para la remediación de suelos contaminados mediante fitoextracción y fitoestabilización;

- Determinar el potencial de producción térmica y de biogás de biomasa vegetal a partir de suelos contaminados con elementos traza para definir su utilidad para la producción de bioenergía.

El trabajo que se presenta en la presente memoria de Tesis Doctoral se divide en tres partes:

1. Revisión de las características químicas y bioenergéticas de los biocarburantes, las principales técnicas y procesos para su producción, así como las características de las distintas materias primas;
2. Recopilación de las técnicas naturales para la recuperación de suelos contaminados con elementos traza, centrada en la fitorremediación;

3. Evaluación de las características químicas y físicas de la biomasa de plantas autóctonas que crecen espontáneamente en suelos altamente contaminados por elementos traza debido a la actividad minera, centrado específicamente en la zona de Cartagena-La Unión, y su potencial uso para la producción de bioenergía (combustión y digestión anaerobia).

Hasta la fecha, la mayor parte de las investigaciones se han centrado en la obtención de biocombustibles de primera generación, en particular: bioetanol (alcohol etílico), producido a partir de residuos y cultivos de alto contenido en azúcares, almidón y lignocelulosa; biodiesel, como un producto del procesamiento de los aceites vegetales obtenidos del cultivo de girasol, canola, soja y otros cultivos oleaginosos; biogás, combustible gaseoso mezcla de metano y CO_2, obtenido de la fermentación de residuos orgánicos de origen animal y vegetal. Los biocombustibles de segunda generación derivan de la conversión de biomasa lignocelulósica a través de la gasificación y la conversión de la celulosa en azúcares. Las ventajas de estos biocombustibles estriban en la facilidad para disponer de las materias primas y las bajas emisiones de gases de efecto invernadero que causa la obtención de las mismas, claramente inferiores a las producidas por los biocombustibles de primera generación. De hecho las plantas herbáceas perennes como el Miscanthus, utilizadas para biocombustibles, pueden cultivarse en suelos pobres en nutrientes no aptos para cultivos agroalimentarios; este tipo de plantas tiene un bajo impacto ambiental debido a la mayor absorción de CO_2 y menor uso de fertilizantes en el cultivo (emisiones GHG en g Ceq MJ^{-1}): Maiz 2,08, Mischanthus 0,512; Rowe et al., 2009).

<u>Biocombustibles de primera generación</u>

El término “biocombustible” se refiere a un combustible en estado líquido o gaseoso obtenido a partir de biomasa (Demirbas, 2009). Tradicionalmente, el uso de biocombustibles se ha vinculado al sector del transporte, para vehículos de motor, en sustitución de combustibles fósiles. Sin embargo, recientemente se ha producido una rápida expansión en la producción y aplicación de biocombustibles para la generación de energía eléctrica y térmica, y en particular, para la cogeneración. Por lo tanto, el concepto actual de biocombustible ha superado el vínculo con el sector del transporte y se ha dado más énfasis a otras aplicaciones. Biocombustibles de primera generación se producen con el uso de tecnologías convencionales, como extracción, fermentación o simples métodos físicos de prensado, a partir de azúcares, almidones, semillas oleaginosas o grasas animales (Tablas 1,2). Por ejemplo, biodiésel a partir de aceites vegetales puros; bioetanol producido a partir de cereales y materias primas azucaradas; bio-ETBE (etil terc-butil éter) producido a partir de bioetanol y biogás de sistemas de digestión anaeróbia (AD). Su producción y aplicación ya existe, siendo los principales márgenes de mejora la reducción de los costes de producción, la optimización del balance energético, el aumento de los rendimientos energéticos de los motores, y el aumento del porcentaje de uso en mezclas con combustibles fósiles. Sin embargo, la producción de biocombustibles de primera generación es limitada porque podría tener un efecto negativo sobre la biodisponibilidad y disponibilidad de alimentos, ya que las materias primas utilizadas también son recursos alimentarios.

Tabla 1. Biocombustibles de primera generación.

Biocombustibles	Composición	Proceso de obtención	Materias primas	Referencias
Biodiesel	Mezcla de ésteres metílicos	Extracción de aceites de semillas oleaginosas, refino y conversión química (transesterificación)	Cultivos de semillas oleaginosas: girasol, colza, soja y aceite de palma	Naik et al. (2010) Rowe et al. (2009) Cencič et al. (2007) Van Gerper (2007)
Bioetanol	Alcohol etílico	Fermentación de los azúcares presentes en la biomasa	***Sacaríferos:*** remolacha azucarera, caña de azúcar, sorgo azucarado. ***Almidón:*** maiz. ***Lignocelulosa:*** sorgo para biomasa.	Naik et al.(2010) Rowe et al. (2009) Chiaramonti (2007)
Bio-ETBE	**Etil-terc-butil-éter**	Compuesto orgánico derivado de alcoholes de etilo e isobutilo	bioetanol obtenido a partir de biomasa o de la parte biodegradable de los residuos	Cencič et al. (2007) Wallace et al. (2009)
Aceites vegetales		Extracción mecánica.	Semillas oleaginosas	Cencič et al. (2007)

Tabla 2. Características químicas de los biocombustibles de primera generación (Cencič et al., 2007).

Características	Biodiesel	Aceites vegetales				Bioetanol	Bio-ETBE
		Girasol	Colza	Soja	Palma		
Valor calorífico inferior (LHV) (MJ kg^{-1})	37	39,6	37,4	36,8	36,5	27	36
Contenido de oxígeno (% peso)	11	10	10,4	10,3	11,5	35	16
Índice de Yodo	108,7	110-143	94-120	117-143	35-61	9	-
Número de Cetano	56	37	32-37,6	36-39	38-42	-	-
Número de Octano	-	-	-	-	-	113	102
Punto de inflamación (°C)	160	274	246	254	-	12	-19
Punto de enturbiamiento (°C)	-2	7,2	-3,9	-3,9	-	-	
Punto de flujo (°C)	-9	-15,0	-31,7	-12,2	-	-	
Viscosidad (cSt)	5,1 (20°C)	37,1 (38 °C)	37 (38 °C)	28,5-32,6 (^{a}38 °C)	8,3 (38 °C)	1,09 (38 °C)	
Estado	Líquido	Líquido	Límpido	Líquido	Límpido	Líquido	Límpido
Apariencia	Límpido	Líquido	Límpido	Líquido	Límpido	Líquido	Límpido

Biocombustibles de segunda generación

Los biocombustibles de segunda generación representan una mejora adicional ya que se obtienen de la biomasa lignocelulósica, e implican mayor grado de innovación que los procesos implicados en la generación de aquellos de primera generación. Se definen como de “segunda generación”, ya que muestran mejores características y rendimiento que los de los biocombustibles de primera generación y, al mismo tiempo, requieren soluciones tecnológicas y de procesamiento más complejas y mejoradas.

Los biocombustibles de segunda generación (Tablas 3,4) se pueden obtener por procesos de conversión termoquímica de biomasa: pirólisis y gasificación. En ellos la biomasa sólida se somete a un aumento progresivo de la temperatura, en ausencia parcial o total de oxígeno, y se producen procesos termoquímicos importantes (oxidación, descarbonilación o descarboxilación), que originan combustibles sólidos (ej. biochar o biocarbón), líquidos (ej. bioaceite o biometanol) o gaseosos (ej. syngas, biogás o biometano), que son la base para el desarrollo de la producción de los biocombustibles de segunda generación. Los procesos a los que se somete la biomasa durante el calentamiento progresivo son los siguientes: secado (150 °C), tostado (entre 150 °C y 270-280 °C), pirólisis (carbonización) (valores máximos de 550-600 °C), y gasificación (a temperaturas entre 600 °C y 1.500 °C).

Dentro de los procesos bioquímicos para la obtención de biocombustibles se incluye la digestión anaerobia para la producción de biogás, que tras su purificación (deshidratación, desulfurización, eliminación de amoniaco, mercaptanos y material particulado) y mejora (eliminación de CO_2) lleva a la producción de biometano, con características similates al gas natural. Otros procesos bioquímicos son la fermentación alcoholica y la transesterificación.

Tabla 3. Biocombustibles de segunda generación.

Biocombustibles	Composición	Proceso de obtención	Materias primas	Referencias
Syngas (o gas de síntesis)	CO, H_2, N_2 y CO_2	Gasificación	Biomasa	Murphy et al. (2004)
Biogás (biometano)	Mezcla de CH_4 y CO_2	Digestión anaeróbica	Estiércol, biomasa de cultivos energéticos, residuos de cultivos, residuos de matadero y de las industrias agroalimentarias, lodo EDAR y fracción orgánica de residuos sólidos urbanos	Braun (2007) Naik et al. (2010)
Biohidrógeno	Hidrógeno	Fotofermentación de ácidos orgánicos, productos intermedios de las vías metabólicas microbianas, mediante la radiación solar.	Biomasa y/o la fracción biodegradable de los residuos	Kotay and Das (2008)
Bioaceite	Aceite	Pirólisis de la biomasa lignocelulósica	Biomasa lignocelulósica	Jahirul et al. (2012) Vivarelli and Tondi (2004)
Biometanol	Metanol	Gasificación de la biomasa y la conversión catalítica de CO_2 y $H_{2,}$a temperatura 400 °C y presión 40-80 atm.	Biomasa lignocelulósica	Cencič et al. (2007) Wakker et al. (2005)
Biodimetiléter (bio-DME)	Dimetil éter	Gasificación de biomasa lignocelulósica a biometanol y su posterior conversión a bio-DME	Biomasa	Cencič et al. (2007) Wakker et al. (2005)
Bio-MTBE (metilterbutiléter)	Metil-ter-butil-éter	A partir de biometanol, eleva el índice de octano de la gasolina, sin disminuir su densidad energética y aumentar su volatilidad.	Biomasa lignocelulósica	Busch et al. (2012)

Biobutanol	Butanol	Fermentación de azúcares por *Clostridium acetobutylicum*	**Sacaríferos:** remolacha azucarera, caña de azúcar, sorgo azucarado **Almidón:** maiz **Lignocelulosa:** sorgo para biomasa	Yusoff et al. (2015)
FT-diésel (FT-líquidos o biocombustibles sintéticos)	Hidrocarburos sintéticos	Síntesis de Fischer-Tropsch: gasificación de la biomasa lignocelulósica, purificación y acondicionamiento del gas de síntesis producido y conversión a biocombustibles líquidos	Biomasa	Naik et al. (2010) Cenčič et al. (2007)

Tabla 4. Características químicas de los biocombustibles de segunda generación (Cencič et al., 2007; Murphy et al., 2004).

Características	**Biogás**	**Biohidrógeno**	**Syngas**			**Bioaceite**	**Bio-metanol**	**Bio-DME**	**Bio-MTBE**	**Bio-butanol**	**FT-diesel**
			Tipo de gasificador								
			aire	**oxígeno**	**vapor**						
Valor calorífico inferior (LHV) (MJ kg^{-1})	23,3	10,05	4,2	10	12	18,5	19,5	28,3	35	36	42.9
Oxígeno (% peso)	trazas		-			45	34,7	35	18	22	
Metano (% peso)	65		2-4	4-6	12,4	-	-		-	-	-
CO_2 (% peso)	40		14-17	25-29	17-19	-	-	-	-	-	-
H_2S (% peso)	0,1		-			-	-	-	-	-	-
Número de cetano	-	-	0,2-1	0,7	2,5	10	5	57	-	17	74
Número de octano	-	130	-			-	104,3	-	110	87	
Temperatura de ebullición (° C)		-252	-			-	65	-23	55	118	
Punto de inflamación (° C)	-	585	-			-	464	292	-	35	315
Estado	gaseoso	gaseoso	gaseoso			líquido	líquido	gaseoso	líquido	líquido	líquido
Apariencia	gaseoso	gaseoso	gaseoso			límpido	límpido	gaseoso	límpido	límpido	límpido

El biogás se produce por digestión anaeróbica a partir de múltiples materias primas, principalmente biomasa vegetal y residuos orgánicos. La digestión anaerobia produce la degradación y la estabilización de la materia orgánica compleja por la acción de los microorganismos en ausencia de oxígeno, que conducen a la producción del biogás rico en CH_4 y CO_2 que se pueden utilizar como fuente de energía renovable para reemplazar las fuentes de energía fósiles (Raposo et al., 2011). Las diferentes etapas del proceso de digestión están definidas por los diferentes microorganismos implicados en los procesos bioquímicos que suceden: hidrólisis, acidogénesis, acetogénesis y metanogénesis. Cada una de las etapas se lleva a cabo por microorganismos específicos. La digestión anaeróbica se considera una fuente competitiva para la producción de energía renovable en términos de eficiencia y costo. Para evaluar la biodegradabilidad anaerobia de un sustrato orgánico como materia prima en biodigestión, se ha utilizado comúnmente una prueba conocida como el potencial de metano bioquímico (BMP) (Angelidaki et al., 2009). El interés actual por el uso de diferentes sustratos orgánicos para la bioconversión anaeróbica está creciendo a nivel mundial, pero no hay referencias claras y comparables debido a los múltiples factores que influyen en el proceso de digestión anaerobia (o biometanización). Además, las diferentes tecnologías varían significativamente los resultados de producción de biogás.

Algunos factores que afectan a la biodegradabilidad y, por lo tanto, a la producción de biogás, incluyen: la materia prima, el tipo y proporción de inóculo y las condiciones de trabajo y tipo de bioreactor. En particular, con respecto a la materia prima, es importante conocer las características del sustrato, tales como humedad, sólidos totales y sólidos volátiles, y especialmente de su materia orgánica, la presencia de polímeros como celulosa, hemicelulosa y lignina, pero también otros compuestos como grasas, fenoles, etc., que van a condicionar la degradación microbiana, y por tanto definir su potencial de generación de biogás (Raposo et al., 2011). De hecho, la biodegradabilidad está limitada por la cristalinidad de la celulosa y el contenido de lignina (Nizami et al., 2009) y la composición elemental. La relación entre el tamaño de partícula y la biodegradabilidad todavía no se ha aclarado, pero para permitir una comparación de los resultados el tamaño de partícula debe ser ≤ 10 mm, y la concentración de sólidos totales < 10 % (Hansen et al., 2004). En cuanto a la influencia del inóculo depende principalmente de seis factores: origen/fuente, concentración, actividad, pre-incubación, aclimatación/adaptación y preservación (Raposo et al., 2011).

La denominada co-digestión se ha desarrollado para mejorar la eficiencia en la producción de biogás y además para introducir en el proceso sustratos de diferentes orígenes, principalmente aquellos considerados como residuos (por ejemplo, residuos agrícolas, estiércol de animales, residuos agroindustriales, cultivos especializados). En co-digestión, los diversos materiales se mezclan en proporciones adecuadas para aumentar la degradabilidad y, por lo tanto, la eficiencia del proceso. Entre los cultivos dedicados, además del ensilado de maíz tradicional, el sorgo azucarado y el triticale parecen ser interesantes. Además, la rotación de cultivos permite una mayor flexibilidad de gestión y mejora la disponibilidad de sustratos durante todo el año (Cencič et al., 2007).

La biomasa vegetal para la producción de biocombustibles ha evolucionado desde los denominados cultivos energéticos (como el maíz) que compiten en recursos con la producción de alimentos, a biomasa forestal y hasta biomasa residual, como restos de poda o residuos agrícolas y agroalimentarios, e incluso biomasa de especies silvestres procedentes de suelos no agrícolas. Por ejemplo, el ***sorgo*** es una alternativa prometedora en el sector de la bioenergía, gracias a sus características agronómicas y adaptabilidad a condiciones limitantes del suelo y del clima. En comparación con el eucalipto, por ejemplo, la producción de materia seca de sorgo es de unas 40 t ha^{-1} en cinco meses frente a las 20 t ha^{-1} en aproximadamente siete años del primero (Pimentel et al., 2017). Hay tres tipos agronómicos de sorgo: sorgo de biomasa, que tiene un alto rendimiento (alrededor de 30 t ha^{-1} de tallo seco) y por esta razón son más adecuados para la generación de biocombustibles sólidos (es decir, combustión directa); sorgo forrajero, cuya biomasa tiene un menor contenido de lignina en los tallos que otros cultivos y, por tanto, es más adecuado para la generación de biocombustibles líquidos; y sorgo de azúcar, a partir del cual se produce bioetanol de segunda generación en rendimentos similares a los de otros cultivos de biomasa como la remolacha azucarera, el maíz o la caña de azúcar. Por lo tanto, el sorgo tiene varios usos potenciales que pueden ser explotados por el sector bioenergético, según el grupo agronómico y parte de la planta utilizada (Pimentel at al., 2017).

Dentro de las especies con mayor futuro para la producción de biocombustibles o bioenergía se encuentra ***Arundo donax***, o caña común. Es una planta herbácea perenne de tallo largo, hueco y robusto que crece incluso en suelos relativamente pobres. Desde su área de origen en la Cuenca del Mediterráneo y Medio Oriente, su distribución se ha expandido a regiones templadas y subtropicales de ambos hemisferios. Es una planta hidrófita capaz de crecer en suelos ricos en agua. Los estudios han demostrado que existen diferencias fenotípicas hereditarias entre los clones de *A. donax* en lo que respecta, por ejemplo, al número, diámetro y altura de los tallos (Cosentino et al., 2006; Pilu et al., 2014). El uso de *A. donax* para la producción de bioenergía se justifica no solo por sus altos rendimientos de biomasa (37,7 t ha^{-1}) sino también porque ha demostrado su aplicación en la producción de bioenergía, tanto a través de la digestión anaerobia con la producción de biogás y mediante combustión directa de su biomasa. Los estudios realizados por Corno et al. (2016) demostraron que *A. donax* puede reemplazar efectivamente al maíz en los procesos de digestión anaerobia, reduciendo los costos de producción, tanto de biomasa como de electricidad.

Recuperación de suelos contaminados

La segunda generación de biocombustibles tiene la ventaja de poder producirse a partir de biomasa lignocelulósica a coste cero, cuando dicha biomasa procede de residuos agrícolas, o de cultivos no alimentarios. Las nuevas tendencias en la obtención de biocombustibles se centran en biomasas generadas en los procesos de recuperación de suelos degradados o contaminados, evitando así la competencia con los cultivos de consumo alimenticio por los recursos naturales (suelo y agua).

El término suelo contaminado se refiere a todas aquellas áreas en las que, como resultado de las actividades humanas, se ha producido la alteración de las características del mismo, y las concentraciones de compuestos o elementos tóxicos superan los límites legales. Los agentes potencialmente contaminantes del suelo están fundamentalmente asociados a residuos derivados de actividades industriales, mineras, agrícolas y ganaderas. Los principales contaminantes de los suelos son:

- Los ***metales pesados***, se producen por deposición atmosférica, la cenizas y escorias de los procesos de combustión de carbón fósil o derivados del petróleo, el aporte directo de actividades agricolas, residuos industriales, urbanos y mineros;

- La acidificación por la ***lluvia ácida***, deposiciones húmedas (agua de lluvia, nieve y niebla) o secas (gases o partículas sólidas) de la atmósfera constituida principalmente por SO_2 y amoniaco, que proceden fundamentalmente de actividades industriales;

- La ***salinización***, como el resultado de la acumulación de sales solubles en la superficie del suelo, que se refleja en un incremento en la conductividad eléctrica de la disolución del suelo con efectos adversos sobre las propriedades físicas y químicas del suelo, que dificulta el crecimiento y la productividad vegetal;

- Los ***fitosanitarios***, como plaguicidas, herbicidas, fungicidas y fertilizantes que se utilizan para progeger los cultivos frente a plagas (insectos, hongos, nematodos, malashierbas, etc.) y enfermedades, con frecuencia formulados con compuestos orgánicos de difícil degradabilidad y con presencia de metales pesados o metaloides cuya aplicación excesiva o prolongada puede llevar a su acumulación en el suelo;

- Los ***compuestos orgánicos*** persistentes dificilmente degradables en el suelo y las aguas, como diversos tipos de hidrocarburos, fenoles, compuestos orgánicos volátiles (COVS), alcoholes, éteres, disolventes clorados, isocianatos, cianuros orgánicos, etc.

- Los **contaminantes emergentes**, como los productos farmaceúticos y de cuidado personal, productos veterinarios, plásticos, microplásticos, aditivos diversos o nanopartículas.

Las técnicas de recuperación de suelos son muy variadas y dependen de un gran número de variables, por lo que es una técnica específica del sitio, tales como: el tipo de contaminante y su concentración, la profundidad de la contaminación; las características del suelo como la composición mineral, el pH del suelo o sus características químicas; las condiciones ambientales e hidrológicas de la zona; la vegetación.

El problema de los suelos contaminados es muy relevante y adquiere una importancia extremadamente alta en el campo del riesgo ambiental y la salud pública. Las áreas sujetas a contaminación y que, por lo tanto, necesitan recuperación son muy numerosas en Europa, e incluyen instalaciones de distribución y almacenamiento de combustible, áreas artesanales e industriales, zonas mineras, vertederos ilegales y áreas de depósito de residuos, etc. Estas áreas son a menudo una fuente de contaminación para el medio ambiente circundante debido a la difusión de los contaminantes mediante la dispersión de partículas de polvo, la lixiviación y la erosión de los suelos (Rodriguez-Eugenio et al., 2018). En Europa existen unos tres millones de sitios potencialmente contaminados, pero no existe ninguna estimación del tamaño real de la contaminación y sus efectos al medioambiente. Se estima que existen más de 137.000 km^2 que necesitan recuperación (Tóth et al., 2016). En España, la política con respecto a la recuperación de suelos contaminados comenzó principalmente en la década de 1990. En la actualidad la Ley 22/2011, de 28 de julio, de residuos y suelos contaminados consistió en la transposición de la Directiva marco de residuos (Directiva 2008/98/CE). Esta ley fue modificada por la Ley 5/2013 de 11 de junio en lo que respecta a la prevención y control integrados de la contaminación. En la actualidad se encuentra en fase de consulta pública un nuevo anteproyecto de ley de Residuos y Suelos Contaminados del 02/06/2020, que pretende transponer dos Directivas comunitarias: Directiva (UE) 2018/851 sobre los residuos, y Directiva (UE) 2019/904, relativa a la reducción del impacto de determinados productos de plástico en el medio ambiente. La nueva Ley revisará y actualizará el régimen jurídico aplicable a los residuos y a los suelos contaminados.

La necesidad de descontaminar grandes extensiones de terreno se ve limitada por el alto costo de las técnicas actualmente disponibles, normalmente procesos de ingeniería química, invasivos y que causan profundas alteraciones químicas, físicas y biológicas en los suelos sujetos a recuperación. A menudo el suelo ya no es adecuado para el cultivo porque durante el proceso de descontaminación toda actividad biológica resulta drásticamente afectada (Mancuso et al., 2004). Para superar estos problemas, se han desarrollado alternativas más baratas y respetuosas con el medio ambiente, como es la fitorremediación (o fitorrecuperación), definido como el uso de plantas para el tratamiento de matrices contaminadas (Salt et al., 1998; Raskin et al., 1999; Pulford and Watson, 2003; Bonomo, 2005).

La fitorremediación se basa en la capacidad de algunas especies de plantas para asimilar, acumular, inmovilizar y degradar contaminantes. Este método de rehabilitación explota la interacción compleja entre el sistema de raíces de plantas y los microorganismos del suelo. La técnica se basa en los procesos naturales realizados por las plantas, que incluyen: absorción directa de metales y algunos compuestos orgánicos; acumulación o transformación de las mismas sustancias químicas por lignificación, metabolización o volatilización; uso de enzimas liberadas por las plantas para catalizar la degradación de compuestos orgánicos contaminantes; liberación de exudados en la rizosfera, que aportan carbono al suelo, modifican el pH y estimulan la actividad microbiana para la degradación de contaminantes.

De acuerdo con los diferentes mecanismos de acción, es posible clasificar las diversas técnicas de fitorremediación en: fitoextracción o fitoacumulación, fitodegradación o fitotransformación, fitoestimulación, fitovolatilización, fitoestabilización o fitoinmovilización y rizofiltración. La elección de las especies de plantas que se utilizarán es una fase crucial en el desarrollo de una estrategia de fitorremediación. El uso de especies nativas del área a recuperar se aconseja especialmente, a fin de evitar la alteración del equilibrio existente en el ecosistema por la introducción de especies exóticas. La elección del tipo de fitotecnología y por tanto de las expecies a utilizar depende del tipo y grado de contaminación y de los objetivos de recuperación (contención, estabilización, aislamiento, asimilación, reducción, desintoxicación o degradación de contaminantes). Dependiendo de estos objetivos, la definición de la estrategia de fitorrecuperación no puede ignorar el conocimiento apropiado de las características del sitio desde el punto de vista edafológico, climático e hidrológico (Bonomo, 2005).

La recuperación de suelos contaminados por elementos traza (metales pesados y metaloides) se presenta como un problema bastante complicado que enfrentar, debido al hecho de que dichos elementos no pueden ser degradados, a diferencia de los contaminantes orgánicos, y permanecen y se acumulan en el suelo (Wade et al., 1993).

La fitoextracción de metales pesados consiste en la absorción de contaminantes a través del sistema de raíces y en la posterior translocación dentro de la planta. Los contaminantes concentrados en los tejidos de la planta se eliminan luego cosechando las plantas, o partes de ellas (Nardella, 2003; Lasat, 2002). La eficacia de la fitoextracción de una planta está determinada por algunos factores: la producción de biomasa; el factor de translocación; el factor de bioacumulación (McGrath and Zhao, 2003). En cambio las plantas adecuadas para fitoestabilización requieren raíces profundas; y baja tasa de transferencia de los contaminantes desde las raíces a las partes aéreas (Rizzi et al., 2004). En el caso de la fitoestabilización o fitoinmovilización, las plantas poseen un mecanismo de tolerancia mediante exclusión, evitando su entrada en sus tejidos a nivel radicular, inmovilizándolo en la rizosfera o reteniendo el contaminante a nivel de raíz, limitando su transporte a la parte aérea.

Una de las limitaciones de los procesos de fitorrecuperación es la eliminación de la biomasa generada, que sin embargo podría ser reciclada utilizándo la para la producción de biocombustibles (Chaney et al., 1997; Kramer, 2005). Esto evitaría un problema muy importante de riesgo de impacto ambiental como la eliminación de biomasa y la destrucción potencial y creciente del hábitat y la biodiversidad, que transformaría grandes áreas agrícolas y convertiría otras áreas, como bosques o pastos, en producción de masa vegetal para biocombustibles.

La combinación de la fitorrecuperación con la producción de biomasa para la obtención de biocombustibles está siendo objeto de estudio en la actualidad, ya que permitiría proporcionar un valor añadido a la biomasa vegetal generada durante el proceso de recuperación, reduciendo los costes del mismo y a la vez promover los objetivos de reducción de combustibles fósiles y de incremento de la energía renovable.

La planificación de una intervención de fitorremediación debe partir siempre de la observación directa del lugar y, sobre todo, de la investigación de las especies presentes de forma natural en el lugar, bien adaptadas a las condiciones climáticas y edafológicas, y que han adquirido determinadas características de tolerancia. La selección de plantas debe tener en cuenta:

- Grado y profundidad de la contaminación. Por ejemplo, la descontaminación de suelos contaminados superficialmente prefiere especies con sistema radicular superficial, propio de cultivos herbáceos; mientras que para una contaminación más profunda se recomiendan especies con sistemas de raíces profundas más desarrolladas como las plantas arbóreas (Gómez et al., 2019);

- Mecanismos de respuesta fisiológica y tolerancia a la contaminación de las plantas.

Las especies de plantas que son particularmente adecuadas para vivir en suelos con un alto contenido de metales pesados se denominan metalofitas. Las especies de plantas se pueden dividir en tres tipos generales en función de su respuesta frente a los elementos traza (ET, metales pesados y metaloides) (Baker, 1981):

- especies acumuladoras, las cuales asimilan los ET del suelo y los concentran en las partes aéreas de las plantas;
- especies indicadoras, aquellas en las que la absorción y el transporte de ET desde las raíces a la parte aérea está controlada y la concentración interna (en la planta) refleja la concentración externa (en el suelo);
- especies exclusoras, las cuales mantienen las concentraciones de ET en la parte aérea (tallos y hojas) en valores bajos y constantes, incluso en presencia de altas concentraciones en el suelo, hasta que se alcanza el valor crítico por encima del cual el mecanismo de control se sobrepasa y se produce una entrada ilimitada del contaminante que provoca toxicidad y muerte. Estas especies tienden a acumular ET en las raíces y limitan el transporte a las partes aéreas (Ali et al., 2013).

La capacidad de las plantas para extraer ET del suelo y transferirlas a sus tejidos se puede evaluar a través de varios parámetros: el factor de bioconcentración (BCF), como la relación entre la concentración total de ET en los tejidos vegetales (parte aérea o raíces cosechables) y la en la matriz o suelo (Conesa et al., 2007), en formas totales o solubles (Mench et al., 2010); la tasa de acumulación (RA), como el contenido total de ET en los tejidos durante el período experimental (Mench et al., 2010); la razón de translocación (TR; también llamado factor de translocación; Conesa et al., 2007), indica la relación entre la concentración total de ET presente en las partes aéreas con respecto a la presente en las raíces (Mench et al., 2010).

Hasta la fecha, se han identificado alrededor de 400 especies hiperacumuladoras, en su mayoría de las familias de Caryophyllaceae, Cyperaceae, Poaceae, Fabaceae, Chenopodiaceae, Asteraceae, pero especialmente Brassicaceae (como *Thlaspi* actualmente *Noccaea* y *Alyssum*). Por ejemplo, *Arabidopsis halleri* acumula Cd (> 100 mg kg^{-1}) y Zn (> 20.000 mg kg^{-1}; McGrath et al., 2006) y *Thlaspi* (Noccaea) *caerulescens* acumula Zn (hojas 100-300 mg kg^{-1}; tallos 10.023-25.561mg kg^{-1}; raíces 3.307- 4.269 mg kg^{-1}), Pb (tallos 4.409-4.706 mg kg^{-1}; raíces 14.485-51.156 mg kg^{-1}) y Cd (tallos 425-1.006 mg kg^{-1}; raíces 174-340 mg kg^{-1}) (Assunção et al., 2003; El Kheir et al., 2008).

Las plantas de gran biomasa pueden ser útiles para la fitoextracción asistida o inducida. Por ejemplo, en *Brassica juncea*, *Helianthus annuus* y *Zea mays* se observó un aumento significativo en la translocación de Pb a las partes cosechables de la planta tras la adición de K_2EDTA al suelo (Magistrelli et al., 2002). En particular, bajo estas condiciones, *B. juncea* mostró una acumulación de Pb en las partes aéreas con valores de hasta 1,5 % de biomasa seca (Huang and Cunningham, 1996; Huang et al., 1997; Blaylock et al., 1997). Al finalizar el proceso de recuperación mediante fitoextracción inducida, es importante planificar el manejo de la biomasa vegetal producida, evaluando el método de disposición más adecuado en relación al tipo y concentración de contaminantes presentes y los requisitos normativos sobre residuos. Para la fitoinmovilización y la fitoestabilización, los contaminantes permanecen retenidos en el sistema radicular y la rizosfera, con un transporte reducido de las raíces a las hojas. Las especies candidatas a realizar fitoestabilización deben presentarse como excluyentes o exclusoras de ET, con un BCF y un TR < 1 (Mench et al., 2010; Mendez and Maier, 2008), con el fin de minimizar la translocación a las partes aéreas y la difusión en el cadena alimentaria. Estas plantas tienen la capacidad de inmovilizar contaminantes en el suelo mediante mecanismos de absorción y acumulación dentro de las raíces, adsorción en la superficie radicular, precipitación en la rizosfera y además promueven la estabilización física del suelo (Suthan Suthersan, 2002).

Utilización de especies fitorremediadoras para la producción de bioenergía

La capacidad como biocombustible de especies de plantas desarrolladas en suelos contaminados se evaluó en condiciones de laboratorio. El objetivo del estudio fue identificar las especies vegetales nativas que tienen características adecuadas para la producción de bioenergía bien mediante combustión o mediante digestión anaerobia y que, al mismo tiempo, pueden tolerar altos niveles de metales pesados y metaloides para su utilización en la recuperación de suelos contaminados mediante las tecnologías de recuperación más prometedoras: la fitoextracción o la fitoestabilización.

Para ello se recogieron diferentes especies de plantas completamente desarrolladas, que crecen espontáneamente en la Sierra Minera de La Unión - Cartagena, como:

Arundo donax L. (Poaceae), *Phragmites australis* L. (Poaceae), *Piptatherum miliaceum* L. Coss. (Poaceae) y *Foeniculum vulgare* Mill. (Apiaceae) de la rambla de El Gorguel (Cartagena); *Dittrichia viscosa* L. (Asteraceae), *Atriplex halimus* L. (Chenopodiaceae), *Bituminaria bituminosa* L. Stirton (Fabaceae) y *Zygophyllum fabago* L. (Zygophyllaceae) de El Llano del Beal (Cartagena).

Las plantas se caracterizaron químicamente respecto a: concentración de macro y micronutrientes y elementos traza por ICP-OES tras digestión ácida en microondas (Tablas 3.1 y 3.2 del Capítulo 3).; sólidos totales (ST) y volátiles (SV); concentración de lignina y holocelulosa (como suma de celulosa y hemicelulosa); análisis elemental de N, C, S, H; y carbohidratos solubles (Tablas 3.5 y 3.6 del Capítulo 3).

Mediante el análisis elemental se calculó el valor calorífico superior (HHV) para determinar su capacidad de generar energía mediante combustión (MJ kg^{-1}). Se realizó un experimento de digestión anaeróbica para determinar el potencial de generación de biogás y el BMP.

Tabla 5. Composición química de las plantas utilizadas en los experimentos (valores medios ± se, n = 2).

Plantas	**Cenizas (%)**	**Sólidos volátiles (%)**	**Holocelulosa (%)**	**Lignina (%)**	**Carbohidratos solubles (g kg^{-1} dw)**	**HHV (MJ kg^{-1})**
D. viscosa	7,03 ± 0,02 c	90,82 ± 0,16 b	50,94 ± 7,21 bc	26,96 ± 0,72 a	54,85 ± 2,95 bc	17,96 ± 0,03 c
A. halimus	14,25 ± 0,14 b	84,14 ± 0,05 c	62,11 ± 5,73 abc	22,40 ± 0,23 b	12,40 ± 0,40 f	16,03 ± 0,04 e
B. bituminosa	5,31 ± 0,06 c	93,19 ± 0,02 a	65,19 ± 0,57 abc	20,09 ± 0,45 bc	26,50 ± 0,80 ef	17,62 ± 0,04 d
Z. fabago	16,28 ± 0,31 a	80,57 ± 0,57 d	48,58 ± 6,07 c	17,08 ± 0,92 c	35,15 ± 0,95 de	15,66 ± 0,01 f
A. donax	6,26 ± 0,19 c	93,74 ± 0,19 a	68,23 ± 0,59 abc	20,92 ± 1,67 bc	83,85 ± 3,15 a	18,75 ± 0,00 a
P. australis	6,81 ± 0,45 c	93,19 ± 0,45 a	72,32 ± 056 a	20,87 ± 0,55 bc	49,50 ± 0,01 cd	18,49 ± 0,01 b
P. miliaceum	7,09 ± 0,61 c	92,92 ± 0,60 a	72,42 ± 0,65 a	18,42 ± 0,33 bc	44,15 ± 3,05 cde	17,87 ± 0,03 c
F. vulgare	6,92 ± 0,33 c	93,08 ± 0,33 a	70,98 ± 0,38 ab	19,59 ± 0,33 bc	69,35 ± 7,35 ab	17,89 ± 0,08 c
ANOVA	***	***	**	**	***	***

** y***: significativo a $P < 0{,}01$ y 0,001, respectivamente. Valores seguidos por la misma letra en cada columna no difieren significativamente de acuerdo al test de Tukey a $P < 0{,}05$.

Tabla 6. Resultados de la degradación anaerobia de las plantas y parámetros del ajuste al modelo cinético de primer orden (media ± se; n=2).

Plantas	**B_m (mL g^{-1}SV)**	**B_0 (mL g^{-1}SV)**	***Kd* (h^{-1})**	**BMP (mL CH_4 g^{-1} SV)**	**TBMP (mL CH_4 g^{-1} SV)**	**Biodegradabilidad anaerobica (%)**
D. viscosa	180,9 ± 3,6 bc	182,2 ± 5,0 bc	0,012 ± 0,001 bc	131,2±3,6 bc	497,9 ± 26,6	26,4± 0,7 bcd
A. halimus	116,6 ± 10,7 c	119,9 ± 14,8 c	0,012 ± 0,004 bc	86,0±10,4 c	576,5± 30,6	15,1 ± 2,9 c
B. bituminosa	224,4 ± 3,2 ab	239,2 ± 0,6 ab	0,011 ± 0,001 bc	177,6±1,1 ab	525,3 ± 6,2	33,8 ± 0,2 abc
Z. fabago	173,8 ± 2,5 bc	184,0 ± 0,1 bc	0,025 ± 0,001 a	130,6±0,04 bc	514,7± 39,9	25,5 ± 2,0 cd
A. donax	261,4 ± 1,5 a	315,9 ± 25,5 a	0,006 ± 0,001 bc	227,4±18,4 a	517,1± 9,8	43,9 ± 2,7 a
P. australis	234,5 ± 9,6 ab	307,0 ± 23,6 a	0,004 ± 0,001 c	218,0±16,8 a	533,5± 9,3	40,8± 2,4 ab
P. miliaceum	277,5 ± 26,9 a	292,3 ± 38,8 ab	0,015 ± 0,003 ab	204,6±27,2 ab	518,9± 2,7	39,5± 5,5 abc
F. vulgare	211,5 ± 16,1 ab	219,4 ±15,3 abc	0,008 ±0,0002 bc	154,6±108 abc	524,3± 2,0	29,5± 2,2 abcd
ANOVA	***	**	**	**	**Ns**	**

** y ***: significativo a *P* < 0,01 y 0,001, respectivamente. Ns = no significativo. Valores seguidos por la misma letra en cada columna no difieren significativamente de acuerdo al test de Tukey a *P* < 0,05.

Resultados y conclusiones

Las diferentes técnicas para obtener bioenergía requieren diferentes características en la biomasa vegetal. Por ejemplo, en combustión directa, la biomasa debe tener un bajo contenido de agua (< 10-12 %) y cenizas y una alta concentración de lignina. De las plantas estudiadas, *A. donax* y *P. australis* poseen características adecuadas (bajos valores de cenizas y altas concentraciones de lignina) para generar energía térmica mediante combustión, con altos valores de HHV, aunque inferiores a los del carbón (22,7 MJ kg^{-1}; Boundy et al., 2011), pero similares a los valores de residuos forestales y de poda de árboles (15,4 y 19,5 MJ kg^{-1}, respectivamente; Boundy et al., 2011).

Los materiales más adecuados para la producción de biogás a través del proceso de digestión anaerobia son aquellos con un alto contenido de materia orgánica. La producción de biogás y el potencial de producción de biogás obtenido en el experimento de digestión anaeróbica fueron generalmente bajos para todas las especies de plantas en comparación con los reportados para cultivos energéticos como el maíz (345 mL g^{-1} en toda la planta), y también para otras especies herbáceas, como la ortiga (210-420 mL g^{-1}), raygrás (360 mL g^{-1}) o girasol (454 mL g^{-1} en toda la planta) (Amon et al., 2007; Braun, 2007; Raposo et al., 2011). Solo los valores de B_0 para *P. miliaceum* y *A. donax* se acercaron a los reportados previamente por Bernal et al. (2019) para especies utilizadas en fitorrecuperación. Los valores de BMP son mayores para las especies de *A. donax* (227,4 mL g^{-1}), *P. australis* (218,0 mL g^{-1}) y *P. miliaceum* (204,6 mL g^{-1}), con valores cercanos a los encontrados para los residuos sólidos derivados de extracción de aceite de girasol (entre 107 y 227 mL g^{-1}; Raposo et al., 2008), y del silo de maíz, cardo y sorgo (267, 308 y 241 mL g^{-1}, respectivamente; Kalamaras and Kotsopoulos, 2014). Pero, los valores obtenidos de las partes vegetativas aéreas de *S. marianum* (174 mL g^{-1}) y *H. annuus* (119 mL g^{-1}) por Hunce et al. (2019) se pueden comparar con los resultados menores obtenidos en este estudio.

La BMP teórica (TBMP) tuvo valores entre 497,9 mL g^{-1} (*D. viscosa*) y 576,5 mL g^{-1} (*A. halimus*), dando una biodegradabilidad anaeróbica promedio entre 15.1 % (*A. halimus*) y 43.9 % (*A. donax*). Solo los resultados obtenidos para *A. halimus, Z. fabago, D. viscosa* y *F. vulgare* son inferiores a los resultados encontrados para restos de poda y madera, también para biomasa de plantas silvestres y restos de cultivos (32,7 - 44,9

%) (Triolo et al., 2012), mientras que para el resto de especies se obtuvieron valores similares o incluso superiores. La baja degradabilidad encontrada para *A. halimus* y *D. viscosa* se puede asociar con el mayor valor de lignina obtenido para la misma especie en comparación con las otras (22,4 y 27,0 % respectivamente). Las especies *P. miliaceum, P. australis* y *A. donax* son las especies con mejores perspectivas para la producción de biogás.

Los datos experimentales considerados en este estudio mostraron que la concentración de ET en las plantas de los suelos mineros no afectó la producción de biogás, ni la producción de energía térmica según el poder calorífico superior. Los resultados obtenidos en la matriz de correlación mostraron que la degradación anaeróbica no estuvo influenciada por la presencia de ET en las plantas (no existieron correlaciones significativas entre los parámetros de digestión anaeróbica y las concentraciones de ET en las plantas; datos no mostrados). Si bien la acumulación de Pb afectó negativamente la degradación anaeróbica de la biomasa de *N. glauca* (225-231 mg Pb kg^{-1} dw), y por tanto la producción de biogás (Bernal et al., 2019). Sin embargo, la concentración de Pb en las plantas estudiadas no alcanzó tales niveles para afectar negativamente la degradación anaeróbica de la biomasa vegetal. Algunos ET han mostrado efectos positivos en la digestión anaeróbica (Demirel and Scherer, 2011); sin embargo, por encima de ciertas concentraciones se vuelven inhibitorias o tóxicas (Zhang et al., 2003). Las concentraciones de ET determinadas en las plantas fueron todas inferiores a esos valores (concentraciones de ET tóxicas en las plantas para digestión anaeróbica: Cd 180, Cu 40, Zn 100 y Pb 30 mg kg^{-1}; Bozym et al., 2015) excepto para Zn. Probablemente, el aporte de ciertos micronutrientes, como Fe y Mn, podría haber estimulado efectos positivos sobre el crecimiento de casi todo tipo de microorganismos en los digestores anaeróbicos (Mudhoo and Kumar, 2013).

Por tanto, las especies más adecuadas para la producción de biogás parecen ser las de la familia Poaceae, y fue la presencia de altas concentraciones de Mg y Na en las plantas (partes aéreas) lo que condicionó la producción de energía como biogás por digestión anaeróbica.

Además, las correlaciones positivas entre los valores de HHV y la producción de biogás sugieren que las especies investigadas pueden utilizarse ventajosamente en general para la fitorremediación de suelos contaminados con ET y luego utilizarse como biomasa para la producción de energía térmica o biogás.

Por tanto, las especies *P. miliaceum*, *P. australis* y *A. donax* son las que poseen mejores perspectivas para la producción de biogás. Sin embargo, *D. viscosa* y *A. halimus* tienen características mas adecuadas para la obtención de bioenergía mediante su combustión directa en lugar de biotransformación anaerobia en biogás, debido a su bajo contenido en cenizas y alta concentración de lignina. No obstante, la presencia de elevadas concentraciones de Na y Mg concentrations en *A. halimus* puede limitar su utilidad en combustión.

Bibliografía

Ali, H., Khan, E., Sajad, M.A. (2013). Phytoremediation of heavy metals - Concepts and applications. *Chemosphere, 91*, 869 - 881.

Amon, T., Amon, B., Kryvoruchko, V., Machmüller, A., Hopfner-Sixt, K., Bodiroza, V., Hrbek, R., Friedel, J., Pötsch, E., Wagentristl, H. (2007). Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. *Bioresource Technol*ogy, *98*, 3204–3212, doi:10.1016/j.biortech.2006.07.007.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., van Lier, J.B. (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Science & Technology,59*, 927-934.

Assunção, A.G.L., Schat, H., Aarts, M.G.M. (2003) *Thlaspi caerulescens*, an attractive model species to study heavy metal hyperaccumulation in plants. *New Phytologist, 159*, 351–360.

Bonomo, L. (2005). *Bonifica di siti contaminati. Caratterizzazione e tecnologie di risanamento*. Milán, McGraw-Hill Education.

Baker, A.J.M. (1981). Accumulators and excluders - strategies in the response of plants to heavy metals, *Journal of Plant Nutrition, 3*(1-4), 643-654, https://doi.org/10.1080/01904168109362867

Bernal, M.P., Gómez, X., Chang, R., Arco-Lázaro, E., Clemente, R. (2019). Strategies for the use of plant biomass obtained in the phytostabilisation of trace-element-contaminated soils. *Biomass and Bioenergy, 126*, 220–230. https://doi.org/10.1016/j.biombioe.2019.05.017

Blaylock, M.J., Salt, D.E, Dushenkov, V., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, Y., (1997), Enhanced accumulation of Pb in indian mustard by soil- applied chelating agents. *Environmental Science and Technology*, *31,* 860-865.

Boundy, B., Diegel, S.W., Wright, L., Davis, S.C. (2011). Biomass Energy Data Book, 4th ed. Oak Ridge National Laboratory, Oak Ridge, US Department of Energy, Tennessee (accessed 17 November 2020). https://info.ornl.gov/sites/publications/files/Pub33120.pdf.

Bozym, M., Florczak, I., Zdanowska, P., Wojdalski, J., Klimkiewicz, M. (2015). An analysis of metal concentrations in food wastes for biogas production. *Renewable Energy, 77,* 467–472. https://doi.org/10.1016/j.renene.2014.11.010

Braun, R. (2007). Anaerobic digestion: A multi-faceted process for energy, environmental management and rural development. In: P. Ranalli (Ed.) *Improvement of Crop Plants for Industrial Uses,* Springer: Dordrecht, The Netherlands, pp. 335–416.

Busch, O., Schade, A., Rasch, H.; Schulte-Koerne, E. (2012). Bio-MTBE. How to reduce CO_2 footprint in fuels with a well known premium gasoline component. In Proceedings of the Conference: “Reducing the Carbon Footprint of Fuels and Petrochemicals”, Berlin, Germany, 8–10 October 2012.

Cencič, V.; Jodice, R.; Migliardi, D.; Picco, D.; Pin, M.; Tomasinsig, E.; Zanotto, G.; Radu, R.; Taccani, R.; Chindaruksa, S. (2007). *I Biocarburanti. Le Filiere Produttive, le Tecnologie, i Vantaggi Ambientali e le Prospettive di Diffusione*, Vol. 27, pp. 26–158. Area Science Park, Padriciano, Italy.

Chaney, R.L., Malik, M., Li, Y.M., Brown, S.L., Brewew, E.P., Angle, J.S., Baker, A.J.M. (1997). Phytoremediation of soil metals. *Current Opinion in Biotechnology*, 8, 279-284.

Chiaramonti, D. (2007). Bioethanol: Role and production technologies. In P. Ranalli (Ed.) *Improvement of Crop Plants for Industrial Uses.* Springer, Dordrecht, The Netherlands pp. 209–252.

Conesa, H., Faz, A., Arnaldos, R. (2007). Initial studies for the phytostabilization of a mine tailing from the Cartagena-La Union Mining District (SE Spain). *Chemosphere, 66*,38–44.

Corno, L., Lonati, S., Riva, C., Pilu, R., Adani, F. (2016). Giant cane (*Arundo donax* L.) can substitute traditional energy crops in producing energy by anaerobic digestion, reducing surface area and costs: A full-scale approach. *Bioresource Technology, 218,* 826–832.

Cosentino, S.L, Copani, V., D’Agosta, G.M., Sanzone, E., Mantineo, M. (2006). First results on evaluation of *Arundo donax* L. clones collected in Southern Italy. *Industrial Crops and Products, 23*, 212–222. https://doi.org/10.1016/j.indcrop.2005.06.004.

Demirbas, A. (2009). Biofuels. In *Biohydrogen. For future engine fuel demands*, Springer-Verlag, London, UK, pp. 61-84.

Demirel, B., Scherer, P. (2011). Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. *Biomass & Bioenergy, 35*, 992-998. https://doi.org/10.1016/j.biombioe.2010.12.022.

El Kheir, S.B., Oubbih, J., Saidi, N., Bouabdli, A. (2008). Uptake and fixation of Zn, Pb, and Cd by *Thlaspi caerulescens*: application in the cases of old mines of Mibladen and Zaida (West of Morocco). *Arabian Journal of Geosciences, 1*(2), 87-95. https://doi.org/10.1007/s12517-008-0007-z.

Gómez, L., Contreras, A., Bolonio, A., Quantana, J., Onate-Sanchez, L., Merino, I. (2019). Phytoremediation with trees. *Advances in Botanical Research*, *89*(10), 281-321.

Hansen, T. L., Schmidt, J. E., Angelidaki, I., Marca, E., Jansen, J. L. C., Mosbæk, H., Christensen, T. H. (2004). Method for determination of methane potentials of solid organic waste. *Waste Management*, *24*(4), 393–400. https://doi.org/10.1016/j.wasman.2003.09.009.

Huang, J.W., Cunningham, S.D. (1996). Lead phytoextraction: species variation in lead uptake and traslocation. *New Phytologist, 134*, 75-84.

Huang, J.W., Chen, J., Berti, W.R., Cunningham S.D. (1997). Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environmental Science and Technology, 31*, 800-805.

Hunce, S. Y., Clemente, R., Bernal, M. P. (2019). Energy production potential of phytoremediation plant biomass: *Helianthus annuus* and *Silybum marianum*. *Industrial Crops and Products*, *135,* 206–216. https://doi.org/10.1016/j.indcrop.2019.04.029.

Kalamaras, S.D., Kotsopoulos, T.A. (2014). Anaerobic co-digestion of cattle manure and alternative crops for the substitution of maize in South Europe. *Bioresource Technology*, *172*, 68–75. https://doi.org/10.1016/j.biortech.2014.09.005.

Kotay, S., Das, D. (2008). Biohydrogen as a renewable energy resource-prospects and potentials. *Internal Journal of Hydrogen Energy, 33,* 258–263. https://doi.org/10.1016/j.ijhydene.2007.07.031

Jahirul, M.I., Rasul, M.G., Chowdhury, A.A., Ashwath, N. (2012). Biofuels production through biomass pyrolysis — A technological review. *Energies*, *5*, 4952–5001. https://doi.org/10.3390/en5124952.

Kramer, U. (2005). Phytoremediation: novel approaches to cleaning up polluted soils. *Current Opinion in Biotechnology, 16*, 133-141.

Lasat, M.M. (2002). Phytoextraction of toxic metals: a review of biological mechanisms. *Journal of Environmental Quality, 31*, 109.

Magistrelli, P., Bregante, M., De Robertis, S., Martell,a L., Paganetto, A., Qualich, P., Racah, J., Natali Sora, I., Gambale, F. (2002), Depurazione di suoli inquinati da metalli mediante fitoestrazione, Istituto di biofisica, CNR, Sezione di Genova, Parco Scientifico Tecnologico della Liguria, Progetto PhyLeS. Available at https://www.scribd.com/document/54790987/2-SUOLO-Metalli-Pesanti-e-Fitorimedio.

Mancuso, S., Pandolfi, C., Azzarello, E. Mugnai S. (2004) . Fitorimediazione, bonificare con le piante. Quaderno informativo n.1 progetto FITOMED, pp. 3 – 12.

McGrath, S.P. Zhao, F.J. (2003). Phytoextraction of metals and metalloids from contaminated soils. *Current Opinion in Biotechnology, 14*, 277.

McGrath, S. P., Lombi, E., Gray, C. W., Caille, N., Dunham, S. J., Zhao, F. J. (2006). Field evaluation of Cd and Zn phytoextraction potential by the hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis halleri*. *Environmental Pollution, 141*(1), 115–125. https://doi.org/10.1016/j.envpol.2005.08.022

Mench, M., Lepp, N., Bert, V., Schwitzguébel, J.P., Gawronski, S. W., Schröder, P., Vangronsveld, J. (2010). Successes and limitations of phytotechnologies at field scale: outcomes, assessment and outlook from COST Action 859. *Journal of Soils and Sediments, 10*(6), 1039 - 1070.

Mendez, M.O., Maier, R.M. (2008). Phytoremediation of mine tailings in temperate and arid environments. *Environmental Science and Biotechnology, 7,* 47 - 59.

Mudhoo, A., Kumar, S. (2013). Effects of heavy metals as stress factors on anaerobic digestion processes and biogas production from biomass. *International Journal of Environmental Science, 10*, 1383–1398. https://doi.org/10.1007/s13762-012-0167-y.

Murphy, M. J., Taylor, J. D., McCormick, R. L. (2004). *Compendium of Experimental Cetane Number Data*. National Renewable Energy Laboratory. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Midwest Research Institute, Battelle, United States. https://pdfs.semanticscholar.org/9ae6/624cba9d45d758d5999756e3e71aba93e667.pdf (accessed, 10 November 2020).

Naik, S.N., Vaibhav, V.G., Prasant, K.R., Ajay, K.D. (2010). Production of first and second generation biofuels: A comprehensive review. *Renewable & Sustainable Energy Reviews, 14*, 578–597. https://doi.org/10.1016/j.rser.2009.10.003

Rowe, R.L., Steet, N.R., Taylor, G. (2009). Identifying potential environmental impacts of large-scale deployment of dedicated bioenergy crops in the UK. *Renewable & Sustainable Energy Reviews, 13*, 271–290. https://doi.org/10.1016/j.rser.2007.07.008.

Nardella, A. (2003). La Phytoremediation. In: La Bonifica Biologica di siti Inquinati da Idrocarburi. EniTecnologie & Eni Corporate University (eds.), Hoepli, Milan, Italy, pp. 87–96.

Nizami, A. S., Korres, N. E., Murphy, J. D. (2009). Review of the integrated process for the production of grass biomethane. *Environmental Science and Technology, 43*(22), 8496–8508. https://doi.org/10.1021/es901533j.

Pilu, R., Cassani, E., Landoni, M., Cerino Badone, F., Passera, A., Cantaluppi, E. (2014). Genetic characterization of Italian giant reed (*Arundo donax* L.) clones collection: Exploiting clonal selection. *Euphytica, 196*, 169–181.

Pimentel, L.D., Batista, V.A.P., de Barros, A.F., Teófilo, R.F., dos Santos Dias, L A. (2017). Chemical and bioenergetic characterization of sorghum agronomic groups. *Pesquisa Agropecuaria Tropical*, *47*(4). https://doi.org/10.1590/1983-40632017v4749170.

Pulford, I. D., Watson, C. (2003). Phytoremediation of heavy metal-contaminated land by trees - A review. *Environment International*, 29(4), 529-540. https://doi.org/10.1016/S0160-4120(02)00152-6.

Raposo, F., Borja, R., Rincon, B., Jimenez, A.M. (2008). Assessment of process control parameters in the biochemical methane potential of sunflower oil cake. *Biomass & Bioenergy,32*, 1235–1244. https://doi.org/10.1016/j.biombioe.2008.02.019.

Raposo, F., De La Rubia, M. A., Fernández-Cegrí, V., Borja, R. (2011). Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures. *Renewable and Sustainable Energy Reviews, 16*(1), 861–877. https://doi.org/10.1016/j.rser.2011.09.008.

Raskin, I., Ensley, B.D. Burt, D. (1999). Phytoremediation of toxic metals: using plants to clean the environment. *Journal of Plant Biotechnology*, *1*(1), 304. https://doi.org/10.1016/S0958-1669(97)80106-1.

Rizzi, L., Petruzzelli, G., Poggio, G., Vigna Guidi, G. (2004). Soil physical changes and plant availability of Zn and Pb in a treatability test of phytostabilization. *Chemosphere, 57,* 10039-1046.

Rodriguez-Eugenio, N., McLaughlin, M., Pennock, D. (2018). *Soil Pollution: a hidden reality*, pp. 142. Rome, Italy: FAO.

Rowe, R.L., Steet, N.R.; Taylor, G. (2009) Identifying potential environmental impacts of large-scale deployment of dedicated bioenergy crops in the UK. *Renewable & Sustainable Energy Reviews, 13,* 271–290,

Salt, D.E., Smith, R. D., Raskin, I. (1998). Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology*, *49*(1), 643–668. https://doi.org/10.1146/annurev.arplant.49.1.643.

Suthan Suthersan S. (2002). Natural and Enhanced Remediation Systems (1st ed.), pp. 440. Boca Raton, Florida: Lewis Publishers.

Tóth, G., Hermann, T.,Da Silva, M.R., Montanarella, L. (2016). Heavy metals in agricultural soils of the European Union with implications for food safety. Environment International, 88, 299-309.

Triolo, J.M., Pedersen, L., Qu, H., Sommer, S.G., (2012). Biochemical methane potential and anaerobic biodegradability of non-herbaceous and herbaceous phytomass in biogas production. *Bioresources Technology, 125*, 226–232. https://doi.org/10.1016/j.biortech.2012.08.079.

Van Gerper, J. (2007). Biodiesel production. In Improvement of Crop Plants for Industrial Uses, Ranalli, P. (Ed.), Vol. 10, pp. 281-290. Dordrecht, The Netherlands: Springer.

Vivarelli, S., Tondi, G. (2004). Pyrolysis oil: An Innovative Liquid Biofuel for Heating. The COMBIO Project. In Proceedings of the International Workshop Bioenergy for a Sustainable Development, Casino Viña del Mar, Chile, 9 November 2004.

Wade, M.J., Davis, B.K., Carlisle, J.S., Klein, A.K., Valoppi, L.M. (1993). Environmental transformation of toxic metals. *Occupational Medicine, 8*, 575 – 601.

Wakker, A., Egging, R., van Thuijl, E., van Tilburg, X., Deurwaarder, E., de Lange, T., Berndes, G., Hansson, J. (2005). Biofuel and bioenergy implementation scenarios. Final Report of VIEWLS WP5, modelling studies (ECN-RX–05-141), p. 105. Petten, The Netherlands: Energy research Centre of the Netherlands. http://www.risoe.dk/rispubl/NEI/nei-dk-4610.pdf.

Wallace, G., Blondy, J., Mirabella, W., Oil, N. (2009). Ethyl Tertiary Butyl Ether – A Review of the Technical Literature. *International Journal of Fuels and Lubricants*, *2*(1), 940-952. https://doi.org/10.4271/2009-01-1951.

Yusoff, M.N.A., Zulkifli, N.W.M., Masum, B.M., Masjuki, H.H. (2015). Feasibility of bioethanol and biobutanol as transportation fuel in spark-ignition engine: A review. *RSC Advances*, *5*, 100184–100211. https://doi.org/10.1039/C5RA12735A.

Zafar, S. (2020) *Biomass Pyrolysis Process*. http://www.bioenergyconsult.com/tag/flash-pyrolysis/ (accessed on 10 November 2020).

Zhang, Y., Zhang, Z., Suzuki, K., Maekawa, T. (2003). Uptake and mass balance of trace materials for methane producing bacteria. *Biomass & Bioenergy, 25*, 427-33.

2. GENERAL INTRODUCTION

2. GENERAL INTRODUCTION

2.1. The use of plant biomass for bioenergy production.

The global demand for production of primary energy is continuously increasing. Consequently, the need to intervene with appropriate measures has arisen in order to achieve economic, social and environmental objectives such as the reduction of fuel and electricity consumption, the containment of atmosphere emissions of greenhouse gases (carbon dioxide, methane and other hydrocarbons) and the improvement of environmental quality in urban centers. In order to meet the growing demand for energy and at the same time address environmental protection issues, the European Union has set itself the goal of increasing the share of renewable energy to 20%, among other objectives of the Europe 2020 Strategy.

The search for efficient measures for the overall improvement of the environment is oriented towards the replacement of traditional fossil fuels by the production of bioenergy (also called green energy). In fact, the different materials and biomass obtained from specific agricultural production and / or plant residues, have physico-chemical and biological characteristics of interest for their use as renewable energy sources. To date, the largest number of investigations and results have focused in first generation biofuels, in particular: bioethanol (ethyl alcohol), produced from residues and crops of sugar, starch and lignocellulose; biodiesel, as a product of oil processing vegetables obtained from the cultivation of sunflower, canola, soybeans and other oilseed crops; biogas, a gaseous fuel made of a mixture of methane and CO_2, obtained from the fermentation of animal and vegetable waste and organic waste. The second generation biofuels are derived from the conversion of biomass through gasification and the conversion of cellulose into sugars. The advantages of these biofuels are the ease of availability of raw materials and the fact that greenhouse gas emissions in obtaining raw materials, are clearly inferior to those of the first biofuels generation (Grippi et al., 2020).

The first chapter of this Thesis (Chapter 1) consist of a review of the existing recent and relevant literature on this regard, with the aim to provide an overview of the chemical and bioenergetic characteristics of biofuels, the main techniques and processes employed for their production, and the characteristics of the different feedstock materials, especially potential energy crops.

2.2. Phytoremediation of contaminated soils: potential for bioenergy production.

Soil pollution is a current major global problem due to the associated risk for both the environment and the public health that it confers. The term pollution generally refers to all the alterations caused in the environment due to the release of harmful substances (so-called contaminants) into the atmosphere, water and soil. Pollution is associated to both the intrinsic toxicity of the contaminant and the release of quantities exceeding the natural self-purification capacity of ecosystems. As a result, the soil physical, chemical and biological characteristics are modified in such a way that is unfavourable to the life of plant and animal organisms (including humans). Anthropogenic pollution is caused by the release of contaminants from different origin: urban biological wastes, agricultural residues or by-products (use of fertilizers and pesticides, animal manure) and industrial activities (mining, production of energy and consumer goods, etc.). The areas that need to be decontaminated include, therefore, fuel distribution and storage sites, craft and industrial areas, mining areas, illegal landfills, waste disposal areas, etc. These areas are often a source of pollution for the surrounding environment due to the spread of pollutants through the dispersion of dust particles, leaching and soil erosion (Rodriguez-Eugenio et al., 2018).

In Spain, the policy regarding the recovery of contaminated sites began substantially in the 1990s. In 1995, the Ministry of the Environment approved the National Recovery Plan of Contaminated Soils (1995-2005), which establishes a series of objectives in management matters and a joint financing mechanism with the Regional Governments, constituting the general framework for the recovery of contaminated sites. In 1998, the Spanish Law on Waste (Ley de Residuos) was approved (Law 10/1998, of April 21, on Waste), and then the law of July 28, 2011 that applies to all types of waste, with the exception of polluting emissions into the atmosphere, radioactive waste and discharges into waterways. One of the fundamental principles of the law was the introduction of a policy of “prevention” of waste, regulation of the production phase and market introduction of products that, once used, generate residual materials.

The Regional Governments must also maintain an inventory of “contaminated soils”, implementing all the necessary procedures for their recovery. In 2005, the implementing regulation was adopted, Royal Decree 9/2005 of January 14, which describes the list of potentially polluting activities in the soil and the criteria and standards for the declaration of contaminated soils (still in effect). The decree uses the category of generic levels of reference (GRLs), among which is the concentration of a contaminant in the soil that does not pose a higher risk than the maximum acceptable risk to human health or for ecosystems. From the GRLs, the Regional Governments may decide to declare a site contaminated in case the GRLs are exceeded or request to run a site-specific risk analysis. But nevertheless, may consider the potential risk low enough not to require more actions. The decree takes into consideration three types of use of the land: industrial, residential and natural. For all three types, different exposure scenarios are considered. In particular, for the three types human-related factors are considered, while only for the third typology the ecosystem is taken into consideration.

In 2009, the National Integrated Waste Plan was approved for the period 2009-2015, part of which is dedicated to contaminated soils. Subsequently, the Law 22/2011, of July 28, on Waste and Contaminated Soils transposes to the Spanish legislation the EU Directive 2008/98/EC on waste, with which has established a comprehensive legal framework in the field of management of waste. The Law 22/2011, which at the same time repealed the previous legislation of 1998, has oriented the waste policy with the principles of maximizing the use of resources, on the one hand, and on the other, minimize the environmental impact, by introduction of measures for the prevention, reuse and recycling of waste, and the promotion of scientific and technological innovation that allows reduce the emission of harmful gases into the atmosphere.

Currently, there are about three million potentially contaminated sites in Europe and over 137,000 km^2 require remediation (Tóth et al., 2016). However, the need of decontaminating large areas is hampered by the high cost of currently available techniques, usually invasive processes that cause profound chemical, physical and biological changes in the soils subject to recovery (Mancuso et al., 2004). To overcome these problems, research has focused on cheaper and more environmentally friendly alternative methods such as phytoremediation (or phytodepuration), defined as the use of plants to treat contaminated matrices (Salt et al., 1998). The selection of the plant species is crucial for successful phytoremediation, as the plants should be able to tolerate contaminants, and to be adapted to the soils and climatic conditions of the area. The cultivation of lignocellulosic biomass crops (mainly native species) in contaminated soils for both phytoremediation and bioenergy production has numerous advantages such as (Bernal et al., 2019): reduction in the competition with food production for bioenergy; low consumption of natural resources (soil and water); compliance with conservation objectives and environmental protection; increased food safety; replenishment of soil C reserves; improvement of soil health; low or zero cost of funding; as well as to avoid the introduction of foreign species into the soil ecosystem to be remediated.

Detailed information regarding soil contamination, remediation alternatives and the use of plant biomass coming from phytoremediation experiments for the production of bioenergy is provided in the second chapter of the Thesis (Chapter 2). An overview of common plants used in the remediation of trace elements contaminated soils together with precise examples of plant species that have shown potential for bioenergy production are summarized in this chapter.

2.3. The Cartagena-La Unión mining district: the use of local species as bioenergy sources.

The use of plants to restore contaminated soils through phytoremediation seems to be a promising alternative as a method to produce biomass for bioenergy. The Cartagena-La Unión mining district is a mountainous formation that extends from east to west along 26 km of coast from the city of Cartagena to the end of Palos Cope, passing through the municipality of La Unión, in the Region of Murcia (Spain). Since ancient times, this area was exploited intensely for the extraction of silver, lead, zinc and other metallic minerals. Mining is one of the main industrial activities that can generate a significant environmental impact, even many years after the closure of the mining activities themselves (Fanfani et al., 2000; Conesa and Schulin, 2010). The Cartagena-La Unión mining district, as well as most of the former mining lands, can be considered suitable for non-invasive remediation technologies, such as phytostabilization (Mendez and Maier, 2008), due to the large surface area that may have to be treated (Conesa et al., 2008).

Several studies have given promising results for phytoremediation with native species belonging to the Asteraceae family such as *Helichrysum decumbens* and *Dittrichia viscosa* (Conesa et al., 2008). Furthermore, spontaneous growth of species such as *Lygeum spartum*, *Piptatherum miliaceum* and *Zygophyllum fabago* (Faz et al., 2008; Conesa et al., 2007) was achieved through the application of organic and inorganic soil amendments, such as pig manure in combination with lime. In addition, different combinations of soil amendments with native species have demonstrated to be useful for the phytostabilization of trace elements contaminated soils of this mining area (Clemente et al., 2019; Pardo et al., 2014, 2017).

The third (and last) chapter of the Thesis (Chapter 3) tries to identify native plant species from the Sierra Minera of Cartagena-La Unión that have characteristics suitable for the production of bioenergy and that, at the same time, can tolerate high levels of potentially toxic TEs, and could therefore be used in the recovery of contaminated soils through Phyto stabilization. The potential for thermal and biogas production of the biomass of plant species growing in TEs contaminated mine soils was determined in order to estimate the usefulness of these species for bioenergy production.

Hypothesis

Through phytostabilization, the establishment of an autochthonous and self-sufficient vegetation cover on contaminated soils could reduce the risk of an uncontrolled transfer of pollutants into the environment, promoting the remediation of the soils. At the same time, these processed can provide useful plant biomass for the production of (bio)energy. This can be considered a suitable and environmentally friendly option to achieve the desired renewable energy share.

Objectives

The aim of this Thesis was to provide relevant, up to date and useful information concerning the bioenergy production potential of plant species that are used for soil remediation by phytostabilization of trace elements contaminated soils. The partial objectives were:

- To review the main techniques of energy production;

-To determine the characteristics of the plant species used for the different biofuels;

- To identify native plant species with characteristics compatible with the bioenergy production which, at the same time, can tolerate high levels of heavy metals and metalloids in the soils where they are grown;

- To review the potential of plant species that grow spontaneously in polluted sites for the remediation of contaminated soils through phytoextraction and phytostabilisation;

- To determine the potential for thermal and biogas production of plant biomass from trace elements contaminated soils to define their usefulness for bioenergy production.

References

Bernal, M. P., Gómez, X., Chang, R., Arco-Lázaro, E., & Clemente, R. (2019). Strategies for the use of plant biomass obtained in the phytostabilisation of trace-element-contaminated soils. *Biomass Bioenergy 126*, 220–230. https://doi.org/10.1016/j.biombioe.2019.05.017.

European Environmental Agency (2005). The European Environment – state and outlook 2020. https://www.eea.europa.eu/soer/2020/soer-2020

Clemente, R., Arco-Lázaro, E., Pardo, T., Martín, I., Sánchez-Guerrero, A., Sevilla, F. & Bernal, M. P. (2019). Combination of soil organic and inorganic amendments helps plants overcome trace element induced oxidative stress and allows phytostabilisation. *Chemosphere 223,* 223–231. https://doi.org/10.1016/j.chemosphere.2019.02.056

Conesa, H.M., Nowack, B., Schulin, R. (2007). A laboratory study on revegetation and metal uptake in native plant species from neutral mine tailings. *Water, Air, Soil Pollut. 183*, 201–212.

Conesa, H.M., Schulin, R. & Nowack, B. (2008) Mining landscape: A cultural tourist opportunity or an environmental problem? The study case of the Cartagena–La Unión Mining District (SE Spain). *Ecol. Econ. 64*, 690–700.

Conesa, H. M. & Schulin, R. (2010). The Cartagena-La Unión mining district (SE Spain): A review of environmental problems and emerging phytoremediation solutions after fifteen years research. *J. Env. Monit. 12*, 1225–1233 |https://doi.org/10.1039/c000346h.

Fanfani, L., Caboi, R., Cidu, R., Cristini, A., Frau, F., Lattanzi, P. & Zuddas, P. (2000). Impatto ambientale dell'attività mineraria in Sardegna: studi mineralogici e geochimici. Rendiconti Seminario Facoltà ScienzeUniversità Cagliari Supplemento, 70, 249-264.

Faz, A., Carmona, D.M., Zanuzzi, A. & Mermut, A.R. (2008). Pig manure application for remediation of mine soils in Murcia province, SE Spain. *Scient. World J. 8,* 819–827.

Grippi, D., Clemente, R., & Bernal, M.P. (2020). Chemical and bioenergetic characterization of biofuels from plant biomass: Perspectives for Southern Europe. *Applied Scence*,10 3571. https://doi.org/10.3390/app10103571.

Mancuso, S., Pandolfi, C., Azzarello, E. & Mugnai S. (2004). Fitorimediazione, bonificare con le piante. Quaderno informativo n.1 progetto FITOMED, 3–12.

Mendez, M.O. & Maier, R.M. (2008) Phytostabilization of mine tailings in arid and semiarid environments—an emerging remediation technology. *Environ. Health Persp. 116*, 278–283.

Pardo, T., Bernal, M.P. & Clemente, R. (2014). Efficiency of soil organic and inorganic amendments on the remediation of a contaminated mine soil: I. Effects on trace elements and nutrients solubility and leaching risk. *Chemosphere 107*, 121-128. https://doi.org/10.1016/j.chemosphere.2014.03.023

Pardo, T., Bernal, M.P., Clemente, R. (2017). Phytostabilisation of severely contaminated mine tailings using halophytes and field addition of organic and inorganic amendments. *Chemosphere*,178, 556-564.

Rodriguez-Eugenio, N., McLaughlin, M. & Pennock, D. (2018). Soil Pollution: a hidden reality. Rome, FAO

Salt, D. E., Smith, R. D. & Raskin, I. (1998). Phytoremediation. *Ann. Rev. Plant Physiol. Plant Mol. Biol. 49*, 643–668. https://doi.org/10.1146/annurev.arplant.49.1.643.

Tóth, G., Hermann, T., Da Silva, M.R. & Montanarella, L. (2016). Heavy metals in agricultural soils of the European Union with implications for food safety. *Environ. Int. 88*, 299-309.

CHAPTER 1. CHEMICAL AND BIOENERGETIC CHARACTERIZATION OF BIOFUELS FROM PLANT BIOMASS: PERSPECTIVES FOR SOUTHERN EUROPE

CHAPTER 1

CHEMICAL AND BIOENERGETIC CHARACTERIZATION OF BIOFUELS FROM PLANT BIOMASS: PERSPECTIVES FOR SOUTHERN EUROPE

1. THE BIOENERGY SITUATION IN EUROPE

The Directive 2009/28/EC established a series of legally binding targets for renewable energy production that has led to a considerable increase in the demand for and production of renewable energy in the European Union (EU). The EU as a whole is likely going to meet its 2020 goals, but some of the states within the EU may need additional efforts to meet their obligations regarding the share of energy derived from renewable sources in the gross final consumption of energy.

Two of the key objectives of the Europe 2020 strategy are a reduction in greenhouse gas emissions of at least 20% compared to 1990 levels and an increase in the share of renewable energy in the final energy consumption of 20%.

Consequently, it is thought that primary production of renewable energy will increase in the long-term (Figure 1). However, despite the growth of new and renewable energy sources, such as solar and wind power, most of the increase in the contribution of renewable energy could be attributable to biomass. In 2015, the production of primary renewable energy in the EU increased by 3.8% compared to 2014 (Eurostat, 2017). For example, electricity production from solid renewables (wood and other solid biomass) used in conventional thermal power stations increased from 3.5% to 7% in 2015. In addition, the production of liquid biofuels has increased significantly, with an average annual growth rate of 32% between 2000 and 2010 (EEA, 2015, 2018).

The year 2018 was a key one for the renewable gas sector in Europe. Several legislative innovations were introduced and adopted by institutions of the Community in order to strengthen its role in the energy transition. New technologies such as power-to-gas and biomass gasification will also be protagonists of this transition. The number of biogas plants in Europe has grown exponentially in the last decade. Between 2009 and 2016, it almost tripled, going from 6200 to 17,662 units, and the growth was particularly intense from 2010 to 2012 (Figure 2). A similar increase was observed in the number of farm-based plants, from 4797 units in 2009 to 12,496 in 2016; such plants are by far the most numerous in the EU, followed by plants that convert sewage sludge (2838), urban waste (1604), and other types of waste (688) (Troussier, 2018).

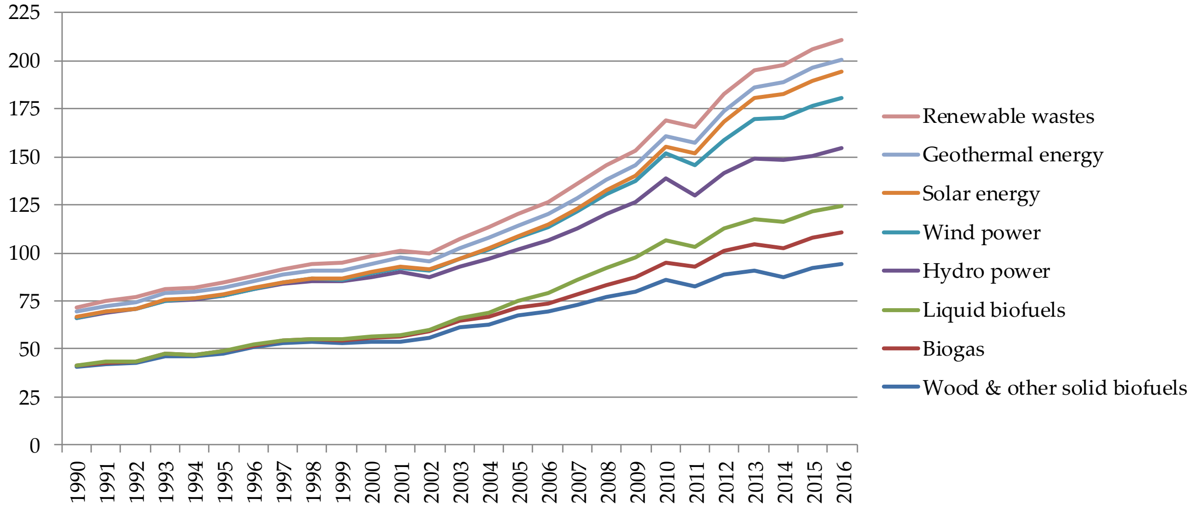

Figure 1. Primary production of energy (Mtoe) from renewable sources, EU—28, 1990–2016 (adapted from Eurostat, 2017; © European Union).

According to Eurostat (Eurostat, 2017), the annual production of biogas in the EU was 181,565 GWh in 2015, with Germany, the United Kingdom, Italy, and France representing the main producing countries. In 2016, the most dynamic countries in the construction of new plants were France (+93) and the United Kingdom (+41). In parallel with the growing maturity of biogas technologies and industry, from 2016 there has been also a substantial increase in the quantity of biomethane produced in Europe. Biomethane is a gas that contains about 97% CH_4 (quality of natural gas) and is obtained from biogas (about 50%–

75% CH_4) after purification (dehydration, desulfurization, removal of gaseous ammonia, mercaptans, dust) and upgrading (removal of carbon dioxide) (Braun, 2007; Adnan et al., 2019). Like biogas plants, biomethane plants (which currently number around 500 units in the EU; Troussier, 2018) mainly use resources from the agricultural sector (380 plants), but also waste (115 plants) and sewage sludge (73). In 2016, EU production of biomethane amounted to 17,264 GWh, with development driven mainly by Germany (+900 GWh), France (+133 GWh), and Sweden (+78 GWh) (Figure 3).

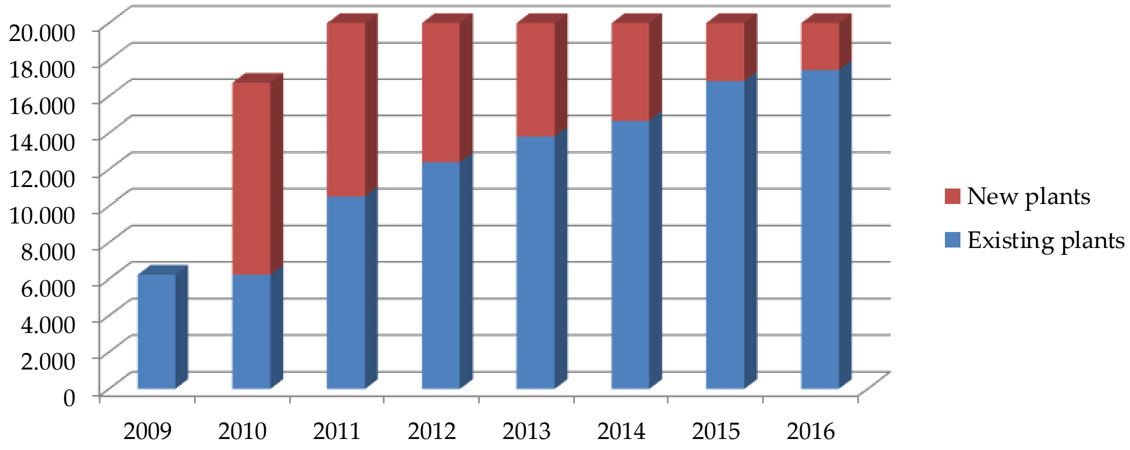

Figure 2. Evolution of the number of biogas plants in Europe (number of biogas plants; adapted from Troussier, 2018).

This growth will likely continue in the next few years, driven by the excellent environmental performance of biomethane and its use as vehicle fuel, and should be immediately achievable by exploiting the existing technologies. The countries that have a large potential for raw materials generation and have defined specific targets for the production of biomethane will consequently experience a significant development in this sector. This is the case, for example, of France, the Scandinavian peninsula, and Italy (Troussier, 2018).

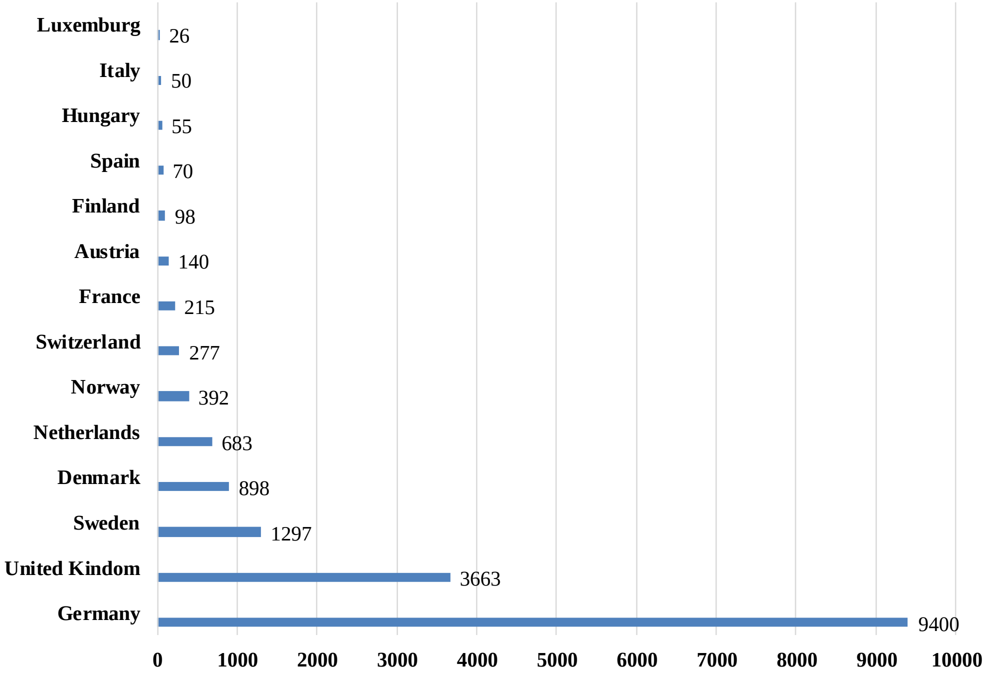

Figure 3. Biomethane production in Europe (GWh) and EU countries active in the production of biomethane (Eurostat, 2017).

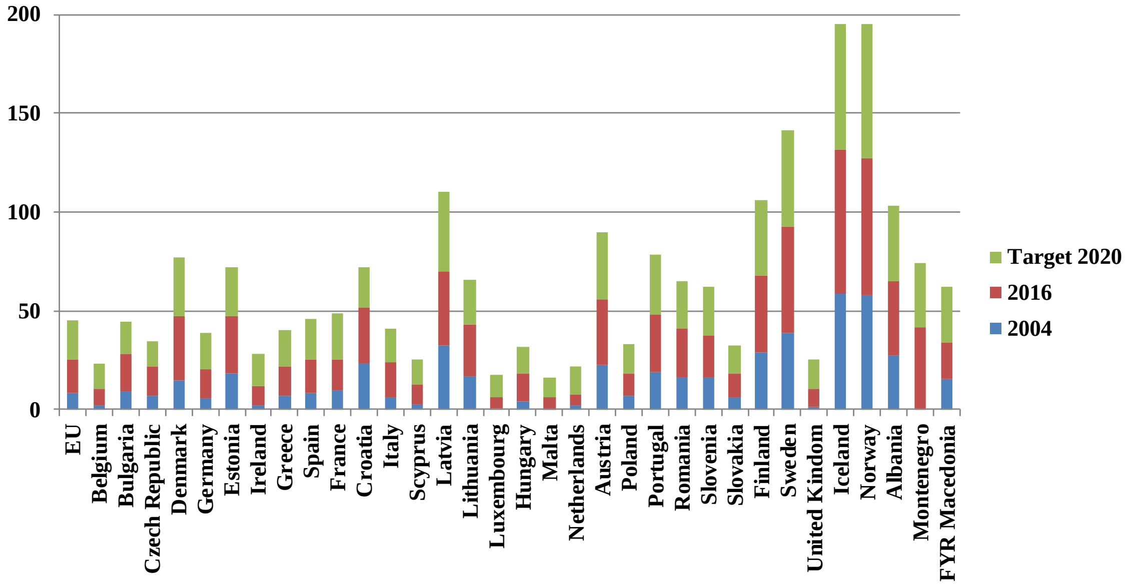

Figure 4. Share of energy from renewable sources in the EU Member states (in % (stacked) of gross final energy consumption; Eurostat, 2017).

Renewable energy (understood as that coming exclusively from sources that replenish themselves naturally) available for final consumption increased by 68% between 2005 and 2015, and by 4.4% in 2015 compared to 2014 (Eurostat, 2017). In the EU-28, the contribution of energy from renewable sources to the gross final consumption of energy increased from 8.5% in 2004 to 16.7% in 2015. This is a clear index of progress towards the Europe 2020 target of 20% (Figure 4). With energy from renewable sources representing more than half (53.9%) of its gross final consumption of energy, in 2015 Sweden had by far the highest share, ahead of Finland (39.3%), Latvia (37.6%), Austria (33.0%), and Denmark (30.8%). Contrastingly, the lowest percentages of renewable energy were registered in Luxembourg and Malta (both 5.0%), the Netherlands (5.8%), Belgium (7.9%), and the United Kingdom (8.2%; Eurostat, 2017). The percentage of the total energy used for heating and cooling coming from renewable energy in the EU-28 increased from 10.2% in 2004 to 18.6% in 2015. In addition, renewable energy will continue to play a key role in helping the EU meet its energy needs beyond 2020, and member states have agreed on a new EU renewable energy target of at least 32% by 2030 and 55%–75% by 2050 (EEA, 2018).

2. BIOFUELS CLASSIFICATION

The term “biofuel” refers to a fuel in the liquid or gaseous state obtained from biomass (Demirbas, 2009). Traditionally, the use of biofuels has been linked to the transport sector, for motor vehicles, to replace fossil fuels. However, there has been a recent and rapid expansion of the production and application of biofuels for electrical and thermal energy generation, and in particular, for cogeneration. Therefore, the current concept of biofuel has outgrown the link with the transport sector, and more emphasis has been given to other applications. Based on the state of maturity of the production and use technologies, biofuels can be divided in two categories (Naik et al., 2010):

1. First-generation biofuels: e.g., biodiesel from pure vegetable oils, bioethanol produced from cereals and sugary raw materials, bio-ETBE (ethyl tert-butyl ether) produced from bioethanol, and biogas from anaerobic digestion (AD) systems. Their production and application has already started, while the main margins for improvement being, at the moment, the reduction of production costs, the

optimization of the energy balance, the increase in the energy yields of the engines, and the increase in the percentage of use in mixtures with fossil fuels;

2. Second-generation biofuels: e.g., bioethanol produced from lignocellulosic raw materials, biohydrogen, syngas, bio-oil, biomethanol, biodimethylether, bio-MTBE (methyl tert-butyl ether), biobutanol, and synthetic diesel, obtained through the Fischer–Tropsch reaction. Their production has not yet started on a commercial scale and is limited to experimental installations. The second-generation biofuels are linked by the possibility of being produced from lignocellulosic biomass at low or zero cost.

This differentiation between first- and second-generation biofuels is important as the former often stand in concurrence with or have undesired economic influences on food production, which implies a series of social/moral and economic consequences that have to be considered (Rowe et al., 2009). First-generation biofuels normally offer some advantages in terms of energy production potential, but they have some disadvantages such as raw materials supply, impact on biodiversity and land use, and competition with food crops, which provokes the increase in food prices resulting from increased production of these fuels (Naik et al., 2010).

However, the production of second-generation biofuels can imply benefits in terms of C-mitigation and ecosystem services, with a demonstrated lower impact on greenhouse gas emissions than the traditional fuels from fossil sources (Rowe et al., 2009). Therefore, second-generation biofuels, produced from plant biomass, one of the most abundant and underused biological resource systems on the planet, can be considered a viable alternative. Biomass, in fact, can be simply burned to produce heat and electricity, or used to produce liquid biofuels. But, as the production of biofuels from agricultural by-products could only meet part of the growing demand for liquid fuels, interest has shifted towards the use of dedicated crops. Second-generation biofuels could, therefore, significantly reduce CO_2 production, not compete with food crops, and in some cases offer better engine performance. If marketed, the cost of second-generation biofuels has the potential to be comparable to standard petrol or diesel and would be the cheapest route to low-carbon renewable energy for transport (Naik et al., 2010).

Comparing the higher heating value (HHV) and lower heating value (LHV) of biofuels with those of fossil fuels, it can be seen that they are normally lower than those presented, for example, by gasoline (HHV = 46.9 MJ kg^{-1}, LHV = 44.2 MJ kg^{-1}), fuel oil (HHV = 43.3 MJ kg^{-1}, LHV = 40.0 MJ kg^{-1}), and diesel fuel (HHV = 45.6, LHV = 42.6). However, the use of biomass provides many environmental benefits (Protásio et al., 2013). The production costs of biofuels are, at present, higher than those of fossil substitutes, even using the most advanced technologies. The cost of producing biogas varies greatly depending on the technology used and, above all, on the raw materials used, which are in most cases self-produced. For these reasons, the definition of the cost is problematic and subject to strong variability depending on the specific situation. In the EU, the optimization of the supply chains and the exploitation of new raw materials allowed a reduction in production costs from 2007 to 2010 of up to 30% (Cencič et al., 2007).

Economic, social, and environmental impacts (including greenhouse gases (GHG) emissions and soil, water, and biodiversity impacts) have been evaluated by IEA (2010). The report indicated, through life cycle assessment (LCA) case studies, that bioethanol production from sugar cane significantly reduces the GHG emissions in comparison with fossil fuels, while the benefits are not clearly demonstrated for other biomass sources. However, clear reductions in GHG emissions were estimated for second-generation bioethanol and biodiesel production from lignocellulose biomass.

2.1. First generation biofuels.

- **Biodiesel** consists of a mixture of methyl esters produced by the chemical conversion of animal and/or vegetable oils and fats and is characterized by a high energy density (37 MJ kg^{-1}; Naik et al., 2010). The materials used for the production of biodiesel are oilseed crops, such as sunflower and rapeseed. Other species, such as soybean or palm oil, are of less interest because their seeds have fairly modest oil contents or they pose problems of environmental and socioeconomic sustainability, respectively (Cencič et al., 2007). The production process includes the extraction of oils from the seeds, refining, and chemical conversion into biodiesel by transesterification reactions (Van Gerper, 2007). The process can be considered energetically efficient, with an output/input energy ratio between 1.25–3.67 for soybean (depending on the production conditions) and 2.29 for rapeseed oil (Rowe et al., 2009), greater than 0.84 considered for diesel oil. Biodiesel is used in thermal motors for the direct production of electrical and thermal energy. However, the biodiesel production sector faces strong competition from the oil production sectors for human consumption of the same resources. Due to some of its properties (like a higher cetane number than gas), biodiesel performs better than diesel, as it shows a greater readiness to ignite and its higher flash point value, compared to diesel, ensures greater safety in handling (Cencič et al., 2007).

- **Bioethanol** is the ethyl alcohol produced by the fermentation of the sugars present in biomass in absence of oxygen and is characterized by a high energy content (27 MJ kg^{-1}) (Chiaramonti, 2007). The raw materials are divided, according to their carbohydrates content, into saccharides (simple sugars), starch, and lignocellulose (cellulose and hemicellulose). Traditionally-used crops are sugar beet in Europe and sugar cane in Brazil as sugar crops (Naik et al., 2010), and maize as a starch crop (Chiaramonti, 2007). The use of lignocellulosic biomass requires different pre-treatments followed by a hydrolysis (acid or enzymatic) step to obtain the monomeric sugars for microbial fermentation (Chiaramonti, 2007). In the case of bioethanol production from starch or sugar crops (wheat grain or sugar beet), hydrolysis, fermentation, and distillation are the most energy demanding steps (64%–74% of total energy input) and involve the greatest GHG emissions, but the use of straw reduces the GHG emissions as this is a waste by-product of grain production (Rowe et al., 2009). Therefore, bioethanol production from by-products is a more environmentally friendly procedure than the production from grain. In terms of energy efficiency, the energy ratio ranges between 1.2 (for maize) to 2.78 for sugar beet (Rowe et al., 2009). But the efficiency depends on the conditions of the industrial process. However, clear advantages in terms of GHG emissions have been revealed by Rowe et al. (2009), with values generally lower that 12 g C eq MJ^{-1}, in comparison with the range found for fuels from fossil sources (18–36.4 g C eq MJ^{-1}).

- **Bio-ETBE** (ethyl-tert-butyl-ether) is an organic compound derived from ethyl and isobutyl alcohols that can be used as an anti-detonator to increase the octane level in gasoline (Wallace et al., 2009). Bio-ETBE is produced from bioethanol and, therefore, shares its raw materials (cereals and sugary raw materials). It is produced through the chemical reaction between isobutanol and bioethanol, with acid catalysis, which takes place on the surface of an ion exchange resin (Cencič et al., 2007).

- **Pure vegetable oils** are obtained by mechanical extraction from oil seeds. Some properties, such as the net calorific value (or lower heating value, LHV), are comparable to those of biodiesel (Cencič et al., 2007).

- **Biogas** is a mixture of the gases carbon dioxide (CO_2) and methane (CH_4), produced as a result of the successive biochemical reactions that take place during the AD process—biomethanation (hydrolysis, acidogenesis, acetogenesis, methanogenesis). The presence of methane in the composition of biogas (50%–75%) (Braun, 2007) has a decisive influence on its energy capacity (average of 23 MJ Nm^{-3}). Pure vegetable oils and biogas are mainly used for the production of electricity and thermal energy generation and cogeneration. Their use in the transport sector is currently limited (Naik et al., 2010).

2.2. Second-Generation Biofuels

Innovative "second-generation" biofuels are obtained from lignocellulosic biomass and represent a promising technological improvement. These biofuels show better characteristics and performance than those of the first generation (Tables 1.1,1.2) but, at the same time, require more complex and improved technological and processing solutions.

- **Biohydrogen** is obtained from the biomass and/or the biodegradable fraction of organic wastes through two different processes: a thermochemical process, which involves gasification followed by a steam-reforming phase to increase the final hydrogen content; and microbial fermentation of sugars in the dark, under anaerobic conditions. Different microorganisms (*Enterobacter*, *Citrobacter*, *Bacillus*, and *Clostridium*) have been reported to produce hydrogen through dark fermentation (Kotay and Das, 2008). This option is similar to AD but is reformulated to produce hydrogen directly instead of methane. While dark fermentation is a major light-independent process, other biological options for biohydrogen production are light-mediated processes, which include direct or indirect biophotolysis and photofermentation. Biophotolysis involves the light-driven decomposition of water in the presence of micro-algae or cyanobacteria, while in photofermentation, photosynthetic microorganisms convert organic acids, intermediate products of the microbial metabolic pathways, into biohydrogen in the presence of solar radiation (Kotay and Das, 2008).

- **Bio-oil** is the liquid product of lignocellulosic biomass pyrolysis, with similar characteristics to petroleum. The pyrolysis process is the initial stage of combustion and gasification processes. Pyrolysis is a thermochemical transformation process at a temperature of about 500 °C, generally carried out in an oxygen-free environment, which yields liquid (bio-oil), solid (biochar), and gas products. Fast pyrolysis is preferred to increase the bio-oil yield, while slow processes are used to obtain biochar (Jahirul et al., 2012). In the fast pyrolysis process, the biomass is rapidly heated to a high temperature in the absence of oxygen, producing about 60%–75% oil products, 15%–25% solid products, and 10%–20% gaseous phase. The characteristics of the fast pyrolysis process are a high rate of heat transfer and heating, a very short residence time of the steam, and rapid cooling of the vapors and aerosol to give a high yield of bio-oil and precision in the control of the reaction temperature. This technology has been widely studied in the last decade because it shows several advantages, like: (i) the production of renewable fuels for boilers, engines, and turbines; (ii) low costs; (iii) low CO_2 production; (iv) the possibility of using second-generation bio-oil and waste materials (forest residues, urban and industrial waste, etc.); (v) ease of storage and transport of liquid fuels; (vi) high energy density compared to combustible gases; (vii) the possibility of separating the minerals, to be recycled as nutrients for the soil; (viii) the possibility of primary separation of sugars and lignin (Jahirul et al., 2012). Bio-oil is one of the highest-quality combustible hydrocarbons, although it is currently burdened by unacceptable energy and economic costs (Vivarelli and Tondi, 2004; Zafar, 2018).

- **Biomethanol** is obtained from lignocellulosic biomass, while traditional methanol is obtained by the catalytic conversion of a fossil fuel (usually natural gas). However, the most widespread process involves the gasification of the biomass and the catalytic conversion (with chromium oxide and zinc oxide) of the CO_2 and H_2 present in the syngas obtained into biomethanol. The reaction for the production of biomethanol generally occurs under conditions of high temperature (400 °C) and pressure (40–80 atm; Cencič et al., 2007). The main problem of biomethanol is linked to the safety of the storage, transport, and handling phases, as it burns without a visible flame and is toxic by inhalation, contact, and ingestion. Moreover, its low volatility (boiling temperature of 78.4 °C, higher than that of petrol, normally around 30–35 °C) leads to problems and higher costs associated with the distribution network (Wakker et al., 2005).

- **Biodimethyl ether** (bio-DME) is dimethyl ether obtained from biomass. The production process is based on the gasification of lignocellulosic biomass to biomethanol and its subsequent conversion to bio-DME. The bio-DME is gaseous at room temperature and liquid at pressures above 5 bar, or if the temperature is below −25 °C. In general, it can be used in the liquid state, operating at pressure values in the order of 5–10 bar (Cencič et al., 2007). DME and BioDME are most commonly used as substitutes for propane in liquid petroleum gas (LPG), especially in Asia, but can also be used as substitutes for diesel fuel in transportation. Besides being able to be produced from a number of renewable and sustainable resources, bio-DME also has the advantage of having a higher cetane number than traditional diesel and, therefore, better combustion quality than diesel fuel during compression ignition. As a result, an engine tailored to work with DME can achieve higher efficiencies, better mileage, and emissions reductions (Wakker et al., 2005).

- **Bio-methyl tert-butyl ether** (bio-MTBE) is produced from biomethanol and has the effect of raising the octane number in gasoline, without reducing its energy density or increasing its volatility. Since isobutene from oil also participates in the synthesis reaction, bio-MTBE is considered a biofuel to the extent that biomethanol is present in its composition (36%). With the gradual elimination of lead, since the mid-1980s it has become one of the most used components for the formulation of gasoline. The lower cost and toxicity of bio-MTBE relative to tetraethyl lead and benzene have increased its use as an anti-knocking agent in all green gasolines. Nowadays, bio-MTBE is used in percentages ranging from 7% to 12% in volume (Busch et al., 2012).

- **Biobutanol** is a liquid biofuel produced, through the fermentation of sugars by the microorganism *Clostridium acetobutylicum*, from the same raw materials as bioethanol (Yusoff et al., 2015). Biobutanol has some positive characteristics compared to bioethanol: it is less corrosive, its mixture with fossil fuels is more convenient because the mixtures do not undergo phase separation, and the storage and distribution of biobutanol are easier. Although biobutanol has a higher energy density than bioethanol, it has a lower octane number and, therefore, better performance (Yusoff et al., 2015).

Fischer–Tropsch diesel (FT-diesel, FT-liquid, or synthetic biofuels) consists of synthetic hydrocarbons or mixtures of synthetic hydrocarbons derived from biomass. The best-known process for the conversion of energy from lignocellulosic biomass into liquid biofuels (biomass to liquid, BTL) is Fischer–Tropsch synthesis, which was used on a large scale in Germany during World War II (Naik et al., 2010). The so-called Fischer–Tropsch process consists of the gasification of the lignocellulosic biomass, the purification and conditioning of the synthesis gas produced (a mixture of carbon monoxide (CO) and hydrogen (H_2)), and its subsequent conversion to liquid biofuels (FT-liquids). The liquid products consist of straight-chain hydrocarbons, do not contain sulfur compounds (which are eliminated in the purification process), and can be converted into fuels for automotive use (Naik et al., 2010). FT-diesel has a behavior similar to that of fossil fuels, in terms of lower calorific value, density, and viscosity, but also a higher cetane number and lower aromatic content, which results in lower emissions of particulate matter and nitrogen oxides. The two fuels can be mixed in any proportion, without the need to make changes in the engine and the distribution infrastructures (Cencič et al., 2007).

-

Table 1.1. Physico-chemical characteristics of first-generation biofuels (adapted from Cencič et al., 2007. © Consorzio per l'AREA di ricerca scientifica e tecnologica di Trieste). LHV: lower heating value; Bio-ETBE: bioethyl tert-butyl ether.

Characteristics	**Biodiesel**	**Vegetable Oils**				**Bioethanol**	**Bio-ETBE**
		Sunflower	**Rapeseed**	**Soybean**	**Palm**		
LHV (MJ kg^{-1})	37	39.6	37.4	36.8	36.5	27	36
Oxygen content (% weight)	11	10	10.4	10.3	11.5	35	16
Iodine number	108.7	110–143	94–120	117–143	35–61	-	-
Cetane number	56	37	32–37.6	36–39	38–42	27	-
Octane number	-	-	-	-	-	113	110
Flash point (°C)	160	274	246	254	-	13	-
Cloud point (°C)	−2	7.2	−3.9	−3.9	-	-	-
Point of flow (°C)	−9	−15.0	−31.7	−12.2	-	-	-
Viscosity (cSt)	5.1 (20 °C)	37.1 (38 °C)	37 (38 °C)	28.5–32.6 (38 °C)	8.3 (38 °C)	0.5 (20 °C)	
State	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Appearance	Limpid	Limpid	Limpid	Limpid	Limpid	Limpid	Limpid

Table 1.2. Physico-chemical characteristics of second-generation biofuels (modified from Cencič et al., 2007. © Consorzio per l'AREA di ricerca scientifica e tecnologica di Trieste). LHV: lower heating value; Bio-DME: biodimethyl ether; Bio-MTBE: biomethyl tert-butyl ether; FT-Diesel: Fischer–Tropsch diesel.

Characteristics	**Biogas**	**Bio Hydrogen**	**Syngas**			**Bio-Oil**	**Biomethanol**	**Bio-DME**	**Bio-MTBE**	**Biobutanol**	**FT-Diesel**
			Type of Gasifier								
			Air	**Oxygen**	**Vapor**						
LHV (MJ kg^{-1})	23.3	10.05	4.2	10	12	18.5	19.5	28.3	35	36	42.9
Oxygen (% weight)	traces			-		45	34.7	35	18	22	
Methane (% weight)	65		2–4	4–6	12.4	-	-		-	-	-
CO_2 (% weight)	40		14–17	25–29	17–19	-	-	-	-	-	-
H_2S (% weight)	0.1			-		-	-	-	-	-	-
Cetane number	-	-	0.2–1	0.7	2.5	10	5	57	-	17	74
Octane number	-	130		-		-	104.3	-	110	87	
Boiling temperature (°C)		−252		-		-	65	−23	55	118	
Flash point (°C)	-	585		-		-	464	292	-	35	315
State	Gaseous	Gaseous		Gaseous		Liquid	Liquid	Gaseous	Liquid	Liquid	Liquid
Appearance	Gaseous	Gaseous		Gaseous		Limpid	Limpid	Gaseous	Limpid	Limpid	Limpid

3. PROCESSES FOR BIOENERGY PRODUCTION

The methods of biomass conversion for the production of energy are classified as thermochemical, biological, or physical (Figure 5).

3.1. Methods of Biomass Thermochemical Conversion

Thermochemical biomass conversion involves drying, roasting, pyrolysis, and gasification. If the solid biomass is subjected to a progressive increase in temperature, in the partial or total absence of oxygen, several thermochemical processes take place, which originate other fuels in solid, liquid, or gaseous form (Naik et al., 2010). Although these methods have been widely used in recent years, thermochemical conversion can be still considered the basis for the development of the second-generation biofuels.

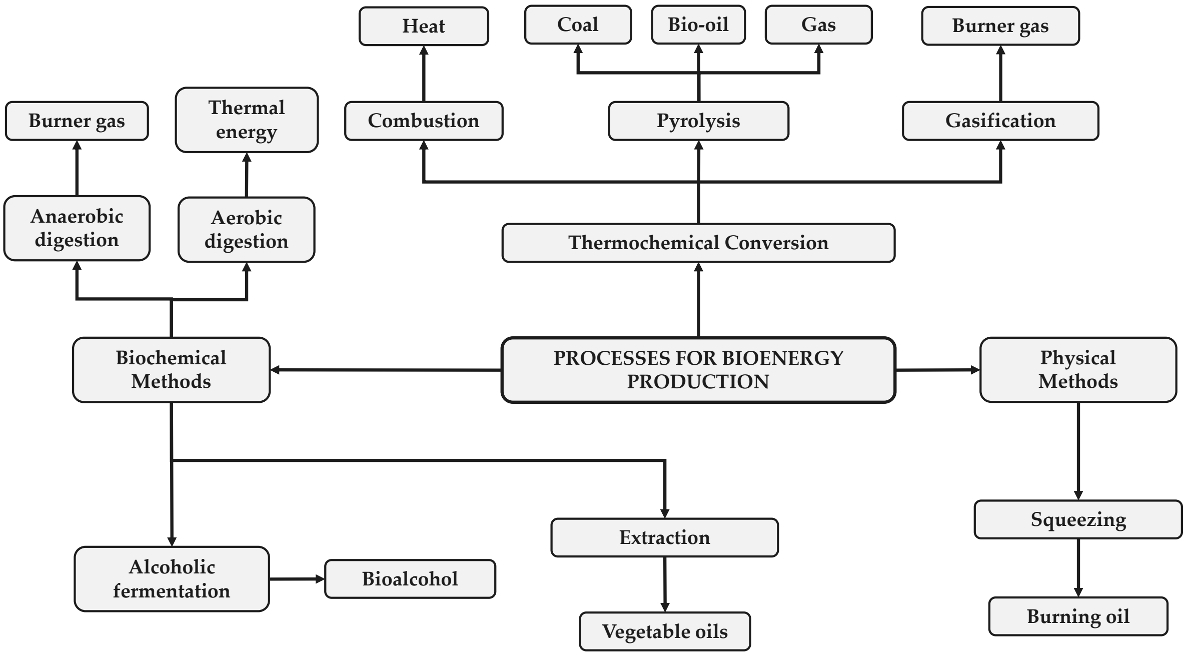

Figure 5. Processes for bioenergy production.

Combustion is the most conventional process. Efficient combustion requires the reduction of the water content of the biomass, generally achieved by open-air drying. Combustion of wet material is less energy efficient as energy is used for evaporation of the water content (Candolo, 2005; Gebreegziabher et al., 2013). For the combustion process to be effective (i.e., compensate the energy used for drying with the improvement in the efficiency of the process), the optimum moisture content of plant biomasses (wood chips, plant residues) has been set to be 10%–15% (Roos, 2008). Pelletizing (the compression and mechanical pressing process) is an effective method to increase density and reduce moisture. Combustion is, from a thermodynamic point of view, a process of conversion of the chemical energy of the fuel (biomass) into heat. The heat is mostly generated by the oxidation of the carbon, in the presence of sufficient oxygen, to CO_2. The biomasses for the production of energy by combustion are generally wood processing residues, and cogeneration is used to produce heat and electricity (Candolo, 2005). A portion of the heat produced is used to produce steam for power turbines connected to electric generators. The remaining heat can be used by industrial or residential users. The efficiency of these plants is in the order of 20%–25% and, therefore, rather modest. Medium–large cogeneration plants can obtain energy efficiencies close to 25%, with about 10 MW of electric power. To generate these powers, the quantities of biomass to be burned become considerable, and complex and costly systems are needed for the elimination of the toxic substances present in the fumes (Candolo, 2005).

According to the temperature reached, the thermochemical processes can be classified as:

- Drying, which occurs at temperatures of up to 150 °C, when water evaporates without substantial chemical modification of the raw material;

- Roasting, which arises between 150 °C and 280 °C, when in addition to water, some organic volatile compounds are released, including acetic acid, methanol, and carbon dioxide. The solid residue darkens, becomes very stable microbiologically, and reaches a high energy density;

- Pyrolysis (carbonization), which takes place when temperatures reach maximum values of 550–600 °C. During this process, the division of the C–C and C–O bonds (decarbonylation and decarboxylation) occurs with the production of gas (mainly carbon monoxide, hydrogen, carbon dioxide, and methane), hydrocarbons (also of high molecular weight, such as tars), phenols, esters, acetic acid, methanol, and water. The resulting solid residue is charcoal (or biochar), while the gas released, if properly cooled, can be divided in two types of product: the non-condensable part and the condensable part, which is liquid at room temperature and is called “pyrolytic juice” or “bio-oil” (Naik et al., 2010). Biochar, with a carbon content greater than 95%, has an LHV of approximately 29.3 MJ kg^{-1}; the energetic value of bio-oil can range between 7 and 18 MJ kg^{-1} according to the water content, while the gas is characterized by an LHV of 4.8 MJ Nm^{-3} (Cencič et al., 2007);

- Gasification, which takes place at temperatures between 600 and 1500 °C, with the total gasification of the biomass. Carbonaceous and organic compounds are converted into fuel gas, also known as syngas, a mixture of mainly carbon monoxide and hydrogen. The syngas can be further converted to hydrogen and CO_2. The high temperatures are obtained by burning aliquots of biomass in the reactor. Air or pure oxygen can be used as a gasification agent: in the first case, the gas produced will contain a high concentration of molecular nitrogen (N_2), while in the second case it will have a lower presence of N_2 but a higher LHV (up to 10.5 MJ Nm^{-3}; Cencič et al., 2007).

3.2. Biochemical Methods

3.2.1. Alcoholic Fermentation

This process is based on the transformation of carbohydrates into ethanol by microorganisms (*Saccharomyces cerevisiae*). The optimal activity of the yeast occurs at 33–35 °C and pH 3–5. The maximum concentration of bioethanol in the growth medium must not exceed 11% by volume, since higher concentrations can inhibit the activity of the yeast (Naik et al., 2010). During continuous fermentation, the concentration of bioethanol is maintained at levels close to the microorganisms’ tolerance values (Cencič et al., 2007).

At the industrial level, fed-batch fermentation and semi-discontinuous fermentation are widely used to fulfill the dual purposes of maintaining the concentration of bioethanol below the threshold for inhibition of yeast and operating under variable volume conditions. In fed-batch fermentation, there is an initial batch phase followed by the fed-batch process once an adequate amount of microbial biomass is reached. This technique is ideal for processes in which either cell growth or product formation is sensitive to the concentration of the limiting substrate (Zohri et al., 2017). Finally, semi-discontinuous fermentation can be considered as a combination of the fed-batch and continuous processes. In this operation, the feed solution is fed at constant intervals, while the effluent is removed discontinuously. The advantage of this system is that the intermittent feeding of the substrate, which has an inhibitory effect on the production of the catabolites, keeps its concentration low, improving fermentation productivity. Both methods have the advantage that there is no non-productive idle time for cleaning and re-sterilization and not much control is required. However, there is a high risk of contamination and mutation due to the long periods of cultivation and handling, and since larger reactor volumes are required, investment costs are higher (Zohri et al., 2017).

Several studies have focused on enhancing the digestibility of lignocellulosic biomass through physical, chemical, biological, and hybrid pretreatments in the production of liquid fuels (primarily ethanol) via biochemical pathways (FitzPatrick et al., 2010; Takara and Khanal, 2011; Sawatdeenarunat et al., 2015.

3.2.2. Transesterification

Transesterification is a chemical reaction in which the main result is the rupture of the triglyceride molecules, i.e., the fatty acids that characterize vegetable oil and are the basis of its high viscosity. The transesterification process takes place using an alcoholic reagent (methanol or ethanol), whose action is reinforced and accelerated by a catalyst (caustic soda). Alcohol, reacting with fatty acids, produces methyl esters (i.e., biodiesel), with a yield of 90% by weight, and glycerol (or glycerin), with a yield of 10% by weight (Naik et al., 2010; Van Gerper, 2007). The splitting of triglycerides, components of oils, into methyl esters has the effect of reducing their viscosity to values close to those of diesel fuel and, as a result, simplifies the interventions necessary for the preparation of the motors, especially for automotive applications. Transesterification can be carried out using different operational temperature and pressure conditions, which are selected in terms of the desired production capacities, the quality of the vegetable oils, and the economic investment that is envisaged (Cencič et al., 2007).

3.2.3. Aerobic Digestion

This process involves the degradation of organic substances by microorganisms under aerobic conditions. The bacteria degrade complex organic substances into simple compounds, with the final production of CO_2, H_2O, and heat, which is produced in proportion to the microbial metabolic activity (Shammas and Wang, 2007), and can therefore be considered a bioenergy production process.

One example of bioenergy production through aerobic degradation is composting. Composting consists of a spontaneous biological decomposition of solid organic material in a predominantly aerobic environment, during which primarily bacteria, fungi, and other microorganisms transform organic materials into a stable product called compost, useful as a fertilizer or soil amendment (Bernal et al., 2017). Composting is an exothermic and spontaneous process in which the microbial activity releases energy as heat, increasing the temperature of the material, which can then be recovered for the production of renewable energy as bioenergy (Onwosi et al., 2017; Smith et al., 2017). Although the ultimate energy available from a composting substrate is the same as that available from the combustion of the substrate, the actual amount of heat produced is determined by factors such as the feedstock energy content, biodegradability, duration of the composting process, and composting conditions (Smith et al., 2017). In fact, composting does not lead to biofuel production, but it can be considered a source of renewable thermal energy. The heat produced from composting can be used directly as vapor for greenhouse heating, as hydronic heating through conduction of heat exchanges inside the pile for consumption of hot water, and by a condenser-type heat exchanger (Smith et al., 2017). The last option is considered the most efficient for energy recovery. Different heat recoveries have been reported from the composting process: 1.9 MJ kg^{-1} of organic matter from tomato plant residues using a rotary drum bioreactor (Alkoaik et al., 2018); 6 MJ kg^{-1} of compost from kitchen and garden waste (Neugebauer, 2018); 17.7–32.9 MJ h^{-1} during the composting of animal manure, animal bedding, and waste feed hay in a commercial-scale system with compost vapor temperatures of 51–66 °C (Smith and Aber, 2018).

3.2.4. Anaerobic Digestion

This biochemical conversion process takes place in the absence of oxygen and entails the microbial degradation of complex organic substances (lipids, proteins, carbohydrates) contained in plants and animal by-products, with the production of gas (biogas). The raw biogas is usually constituted mainly of methane (about 50%–75%), CO_2 (25%–50%), H_2O (1%–5%), and other minor volatile components, such as N_2, H_2S, NH_3 and siloxanes or other organic hydrocarbons (Braun, 2007). During AD, degradation and stabilization of complex organic matter are performed by microorganisms, leading to the production of an energy-rich biogas that can be used as renewable energy to replace fossil energy sources (Raposo et al., 2011; Angelidaki et al., 2011).

The digestion of the biomass takes place in four phases (Figure 6): hydrolysis, acidogenesis, acetogenesis, and methanogenesis, each one carried out by specific microorganisms (Braun, 2007). In the hydrolysis phase, complex organic substrates, such as proteins, fats, and carbohydrates, are degraded by different bacterial groups, with the formation of simple compounds, such as amino acids, fatty acids, monosaccharides, and alcohols. The acidogenic phase oxidates microbially the hydrolyzed simple organic substrates to low molecular weight intermediate compounds (e.g., lactic, propionic, butyric, formic, and acetic acids, ethanol, and H_2), which are converted into acetate in the subsequent acetogenic phase by acetogenic bacteria. Then, methanogenic archaea are responsible for the final degradation and the formation of CH_4 (Raposo et al., 2011).

Normally, the biogas produced is used in an endothermic engine to produce electricity. The electricity produced can be directly fed into the distribution network and sold at a remunerative price as a renewable energy source. Frequently, the digested material is applied to agricultural soil as a source of nutrients (due to its high concentration of nutrients, mainly N as NH_4^+ that is directly available to plants), partially substituting mineral fertilizers (Alburquerque et al., 2012). The most widely used biomass is maize silage (about 10 m^3 of methane can be obtained from 1 t of maize silage; Candolo, 2005). However, other organic materials are also used frequently as feedstocks for AD, like animal manure, crop residues, sewage sludge, industrial wastewater, food waste, and municipal solid waste (Braun, 2007; Sawatdeenarunat et al., 2015).

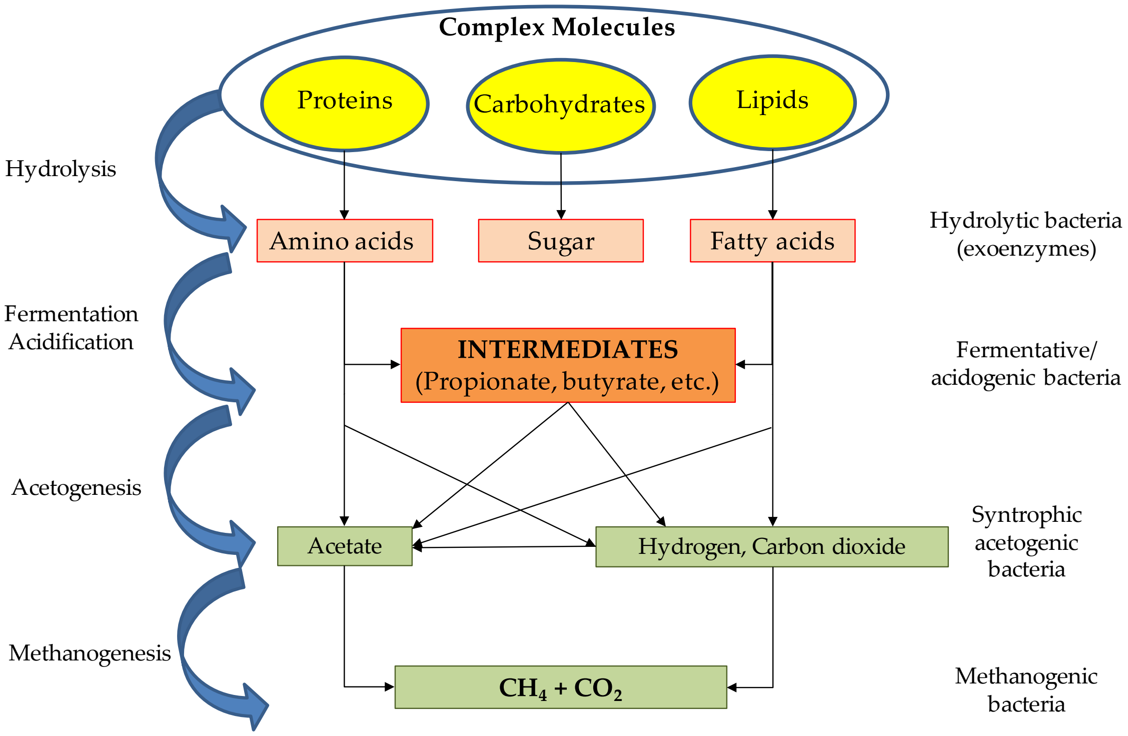

Figure 6. Phases of the anaerobic digestion process (adapted from Naik et al., 2010. © Elsevier Ltd).

The calorific value of the biogas obtained varies according to the methane content. A typical value can be about 23,000 kJ Nm^{-3} (Cencič et al., 2007). The biogas can be treated, accumulated, and used as fuel for gas boilers or internal combustion engines. However, it can also be purified (i.e., removal of CO_2, H_2O, H_2S- or S-containing compounds (mercaptans), siloxanes, and other minor components) (Braun, 2007; Deublein and Steinhauser, 2008) and upgraded to produce biomethane (97% CH_4), with a quality equivalent to natural gas, which can be used as a biofuel for transport and for introduction into the natural gas network. Upgrading the biogas by removing CO_2 and increasing the CH_4 concentration increases the energy content of the biogas, but also the costs of biogas production. Some technologies for biogas upgrading are already available, and others are in the pilot or demonstration plant stage (Petersson and Wellinger, 2009). Four methods are in use for biogas upgrading through CO_2 removal: washing on a temperature or pressure swing, in which a liquid removes CO_2; cryogenic, based on cooling and compressing for removing liquid CO_2; vacuum pressure swing adsorption (VPSA), in which a packed bed absorbs CO_2; selective membranes, which permeate through the CO_2

or methane, depending on the applied membrane (Kampman et al., 2016). Anaerobic digestion is, therefore, considered a competitive source for the production of renewable energy in terms of efficiency and cost (Braun, 2007; Raposo et al., 2011). Considering the energy requirement of the process (input) and the energy produced (output), the output/input ratio reveals a high efficiency for crop residues such as potatoes, fodder beet, and maize (4.6–5.1), with low efficiency for rye and rapeseed oil (1.4 and 2.1, respectively) (Braun, 2007).

More recently, lignocellulosic biomasses, namely agro-residues and energy crops, have been gaining much attention as candidate feedstocks for the generation of bioenergy and biobased products (Amon et al., 2007; Braun, 2007; Oslaj et al., 2010). Unlike conventional biorenewable feedstocks (i.e., sugar- and starch-rich crops), lignocellulosic biomass does not directly compete with food or feed production (Sawatdeenarunat et al., 2015). The composition of lignocellulosic biomass, however, consists primarily of cellulose, hemicellulose, and lignin, and the combination of these components creates a highly resistant and recalcitrant biomass structure. Consequently, the hydrolysis of lignocellulose often becomes the rate-limiting step during traditional AD (Khanal, 2008). In any case, lignocellulosic biomass has a great potential to serve as a feedstock for CH_4 production. The complexity of the biomass structure and the lack of appropriate digesters designed for efficient handling of the high-solids biomass are major challenges in the digestion of lignocellulosic biomass. However, harvesting of the biomass at an appropriate stage of maturity and co-digesting it with other feedstocks significantly enhances the CH_4 yield (Sawatdeenarunat et al., 2015).

A common alternative biological pretreatment of feedstocks is treating them with rumen microorganisms (RM), the microflora found in ruminant stomachs, which create an in situ cellulolytic ecosystem that has a high potential to degrade the complex carbohydrate structures of lignocellulosic biomass (Hu and Yu, 2005). Anaerobic degradation using RM is a pH-dependent process. The highest efficiency in terms of substrate digestion was found at a pH between 6.8 and 7.3 (Hu et al., 2004).

To evaluate a specific organic substrate for biogas production through AD, the biochemical methane potential (BMP) test is commonly used (Angelidaki et al., 2009).

The general principle is the anaerobic incubation of the organic substrate in an inoculum containing a suitable medium (water and minerals) with a variety of anaerobic microorganisms, at neutral pH and with specific temperature conditions (mesophilic or thermophilic). The substrate acts as a source of carbon and energy for the microorganisms. During the incubation, the production and composition of the biogas and the degradation rate of the substrate are evaluated and the conversion rate determined (Raposo et al., 2011). The determination of the BMP allows the definition of the potential of a material to produce biogas by AD on an industrial scale. However, the efficiency of the industrial process must be proven.

The factors that affect the biodegradability and, therefore, the production of biogas include: the characteristics of the input materials (composition of the organic matter, moisture, total solids and volatile solids contents, biodegradability, and even the crystallinity of the cellulose) and the inoculation and anaerobic conditions (Amon et al., 2007; Lehtomäki et al., 2008; Nizami et al., 2009; Oslaj et al., 2010; Plöchl et al., 2009; Raposo et al., 2011).

In general, AD is classified into three important groups based on the operating total solids (TS) content: liquid (L-AD), with TS < 10%; semi-solid (S-AD), with 10%–20% TS; and solid-state (SS-AD), with TS > 20% (Cui et al., 2011; Karthikeyan and Visvanathan, 2012). However, Brown et al. (2012) referred to liquid and solid-state AD with TS content < or >15%, respectively. SS-AD is ideal for high-solids organic feedstocks, which typically have a TS content between 10% and 50%, like energy crops, food wastes, livestock manure, agro-residues, etc. (Lehtomäki et al., 2008). In recent years, there has been growing interest in SS-AD with agricultural and forest residues, and also with energy crops. The advantages of SS-AD, in comparison to L-AD, are: lower reactor volumes, higher organic loading rates (OLRs), less water for dilution, a lower mixing requirement, no floating substrates (in the bioreactor), lower costs for managing the digestate, and overall, a lower energy input for operation (Karthikeyan and Visvanathan, 2012; Liew et al., 2012). Typically, due to the higher OLR during SS-AD, the accumulation of volatile fatty acids (VFAs), organic acids, and ammonia leads to an overload of the reactor and the whole system is prone to fail, especially when energy crops are used as a mono-substrate. Thus, the high solids content in SS-AD, which is considered an advantage, can also produce dead zones in the reactor and can lead to reductions in CH_4 production efficiency, as well as causing an overload of the reactor. Therefore, in this regard, further research is needed on reactor configurations to eliminate the build-up of toxic and/or inhibitory compounds in SS-AD systems. The efficiency of SS-AD systems can be further improved through different reactor operating strategies, adequate OLRs and retention times, maintenance of healthy and well-balanced microbial communities, and subsequent identification and quantification of the efficiency of lignocellulosic-biomass-digesting microbes (Sawatdeenarunat et al., 2015). A possible future path for SS-ADs could be the two-step co-digestion of energy crops and animal manure. This approach could improve the microbial hydrolysis of substrates, prevent VFA build-up, and provide the necessary nutrients and trace elements. It would also be interesting to study and understand how the maturity and composition of animal feed affect the digestibility of biomass (Sawatdeenarunat et al., 2015).

The AD of N-rich substrates, such as animal manures, is also not recommended as it can result in digester instability caused by ammonia toxicity from the rapid degradation of organic nitrogen such as urea and protein (Abouelenien et al., 2014). In co-digestion processes, substrates from different origins (e.g., animal manures, agroindustrial wastes, crop residues, etc.) are mixed to increase their degradability and also to adjust the C/N ratio and, therefore, the efficiency of the process. The C/N ratio of feedstocks is critically important to facilitate the conversion of lignocellulosic biomass to CH_4 (Wu et al., 2010; Giuliano et al., 2013; Ye et al., 2013).

Thus, co-digestion of carbohydrate-rich lignocellulosic biomass with nitrogen-rich animal waste has significant implications for the maintenance of an optimal C/N ratio for commercial CH_4 production with renewable feedstocks (Braun, 2007; Giuliano et al., 2013). Several studies to date have demonstrated the successful anaerobic co-digestion of livestock wastes and lignocelluloses. Ye et al. (2013) reported the co-digestion of rice straw and swine manure in a series of batch experiments where co-digestion increased the CH_4 yield by an impressive 71% compared to the mono-digestion of rice straw, using a pig manure to rice straw ratio of 2:1 (on a vs. basis). The C/N ratios of the co-substrates (i.e., the mixture of swine manure and rice straw) and mono-substrate (i.e., rice straw) were 21.7 and 47, respectively.

3.3. Physical Methods

Physical conversion is essentially a process of mechanical pressing of crops with high oil content (like sunflower, rapeseed, and soybean). The vegetable oil obtained, which is a mixture of fatty acid esters, can be used directly as fuel for slightly modified diesel engines or can be chemically treated to make it similar to diesel fuel (biodiesel; Candolo, 2005). The oils are normally extracted from the seeds of plants through cold or hot mechanical extraction methods, similar to those for producing oil for human consumption. They consist of a screening and press or centrifuge system, filtration (with carbon filters, plate filters, or centrifugal filters), and storage in tanks. The operational conditions can be continuous, taking place between the inner walls of a cylindrical chamber with a mechanical element (generally a screw) inside, in rotation on its longitudinal axis, or discontinuous, run with compression cycles (Ciaschini et al., 2005). During the extraction, the temperature can increase due to the friction occurring between the seeds and the press, but the temperature can also be controlled by a thermoregulation system. Average oil yields are 30%–33% by weight, but by optimizing the parameters that regulate the process, yields can reach 36% by weight. In fact, both the pressure and the temperature of the extraction significantly affect the yield; the influence of the pressure seems to be, however, more important than that of the temperature (Ciaschini et al., 2005).

4. CHARACTERISTICS OF THE SOURCE MATERIALS FOR BIOENERGY PRODUCTION

Among the different renewable energy sources, plant biomass is the most versatile from which to obtain solid, liquid, or gaseous fuels, and, in some cases, it can be directly used as a fuel (Iaboni and De Stefanis, 2003). It constitutes, therefore, a renewable and inexhaustible resource—if properly used—for the production of (bio)energy.

Conventionally, biomass can be classified into three sectors:

- Lignocellulosic materials, which include biomass crops and crop residues;

- Biofuel crops, which are subdivided into oilseed crops, such as rapeseed and sunflower, and sugary crops, such as sugar cane; and

- Organic materials, from which biogas can be obtained through fermentation or degradation (Iaboni and De Stefanis, 2003).

The biomasses suitable for energy transformation, whether it takes place directly using the biomass or after its transformation into a solid, liquid, or gaseous fuel, can also be subdivided by category of origin into the following types:

- Forestry and agroforestry: residues from silvicultural or agroforestry activities, use of coppice woods, etc.;

- Agriculture: crop residues deriving from agricultural activity and dedicated crops of lignocellulosic species, oleaginous plants (for the extraction of oils and their transformation into biodiesel), and alcohol plants (for the production of bioethanol);

- Livestock waste: for the production of biogas;

- Industrial: waste from wood or wood products and the paper industry, as well as residues from the agri-food industry;

- Urban waste: residues from public gardens, maintenance operations, and the wet fraction of municipal solid waste.

Table 1.3. Chemical composition of energy crops.

Crop	Carbohydrate (% d.m.)	Fat (% d.m.)	Protein (% d.m.)	Ash (% d.m.)	Dry Matter (t ha^{-1})	Moisture (%)
Oilseed crops						
Rapeseed	17.0 [a]	41–50 [b]	24.7 [a]	4.3 [a]	7.5 [a]	9–12 [c]
Sunflower	15.9 [a]	48–55 [b]	25.2 [a]	4.2 [a]	4.0 [a]	9 [c]
Soy	31.8 [a]	18–21 [b]	37.9 [a]	5.2 [a]	5.4 [a]	
Abyssinian mustard	30–38 [q]	30–39 [b]	38.9 [q]	5.2 [q]	-	8.9 [q]
Alcohol crops						
Sugary						
Sugar beet	Roots 18 [b] (sucrose. glucose, fructose)	Leaves 2.7 [e] Pulp 0.2 [e]	Leaves 16 [e] Pulp 1.00 [e]	Leaves 32 [e] Pulp 0.50 [e]	30–40 [d]	-
Sugar sorghum	Stems 15 [b] (sucrose, glucose, fructose)	2.0–2.4 [f]	8.6–9.4 [f]	Panicle 7.85 [g] Leaf 9.44 [g] Stalk 4.38 [g] Bagasse 3.41 [g]	25.29 [g]	Panicle 36.43 [g] Leaf 60.58 [g] Stalk 66.40 [g] Bagasse 47–56.62 [g]
Starchy						
Triticale	13 (Starch) [e]	3.23 [e]	8.6 [e]	6.01 [e]	16.5 [d]	50 [c]
Forage sorghum Grains	11 [b] (cellulose) 18 [b] (hemicellulose) 30 [b] (starch)	1.7–2.3 [f]	8.2–9.9 [f]	Panicle 4.19 [g] Leaf 9.22 [g] Stalk7.19 [g]	21.37 [g]	Panicle 49.80 [g] Leaf 60.45 [g] Stalk 71.26 [g] Bagasse 46.50 [g]
Maize	Grains 70 [b] (starch)	1.9–2.6 [f]	6.6–8.6 [f]	Bagasse 1.1 [r]	21.5 [d]	27.7–33.9 [f]

Table 1.3. Chemical composition of energy crops (cont.).

Crop	Carbohydrate (% d.m.)	Fat (% d.m.)	Protein (% d.m.)	Ash (% d.m.)	Dry Matter (t ha^{-1})	Moisture (%)
			Lignocellulosic			
Biomass sorghum	--	1.6–3.3 [f]	7.8–10.2 [f]	Panicle 4.34 [g] Leaf 6.30 [g] Stalk 4.77 [g]	42.33 [g]	Panicle 52.79 [g] Leaf 47.90 [g] Stalk 66.22 [g]
Common cane	31 [b] cellulose 22 hemicellulose	0.8–1	1.3–3.7 [h]	Leaves 11.3 [d] Stems 3.2 [d]	37.7 [d]	
Miscanthus	41 [l] cellulose 24 [l] hemicellulose	-----	1.0–2.2 [h]	Leaves 6.2 [d] Stems 2.9 [d]	15–30 [d]	31 [i] average of two years
Cardoon	41 [m] cellulose 23.6 [m] hemicellulose	0.1	2.9–3.7 [h]	6.8–8.2 [h]	0.4–24.8 [h]	19.1–55.5 [h]
Switchgrass	63.2 [n]	4.0 [n]	12.8 [p]	Leaves 7–7.6 [d] Stems 2.3-2.6 [d]	10–25 [o]	35–45 [o]

[a] (Santonoceto, 2014); [b] (Cencič et al., 2007); [c] (Mancini et al., 2011); [d] (Corno et al., 2014); [e] (Calabrò, 2018); [f] (Giunco, 2015); [g] (average of 2 sorghum cultivars, modified from (Calabrò, 2018); [h] (Mantineo et al., 2009); [i] (Giovanardi et al., 2009); [l] (Consentino et al., 2007); [m] (Palumbo and Mastro, 2014); [n] (Petrini et al., 2005); [o] (Saccani et al., 2015); [p] (Anaborapi, 2001); [q] (average of 4 *Brassica carinata* cultivars from (Xin et al., 2013); [r] (Demirbas, 2004).

Depending on the type of biomass used and the type of processing used, it is possible to classify biomass in two groups: that produced specifically for energy purposes (dedicated crops) and that recovered from the agricultural, forestry, and agroindustrial sectors (recovery biomass). The dedicated crops can be classified in terms of the specialization of production or of the physico-chemical characteristics of the cultivated species to which the transformation process is inevitably linked (Table 1.3). In fact, dedicated crops can be divided into three main categories: lignocellulosic biomass crops, oil crops, and alcohol crops (Marrone, 2014).

Studies carried out to date have shown that the efficacy of the different processes of energetic transformation, such as direct combustion, conversion into bioethanol, and the gasification of lignocellulosic crops, depends not only on the physico-chemical properties of the different plant species but also on the different parts of the plant (stems, leaves) used. For example, for direct combustion the biomass at harvest must have low water and ash content and a high amount of raw lignin; whereas, in the production of ethanol or in gasification systems, the lignocellulose components (such as cellulose and hemicellulose) are important (Consentino et al., 2007). In AD, if traditionally the materials used were mainly animal manure or slurry, today co-digestion processes are favored (Abouelenien et al., 2014; Giuliano et al., 2013; Wu et al., 2010; Ye et al., 2013). Co-digestion can achieve a significant enhancement of the biogas yields and, therefore, of the energy produced, as well as greater stability of the digestion process (Cavinato et al., 2010; Cencič et al., 2007). Silage maize is the most widely used material, both for its high energy and crop yield (at least in particularly suitable areas) and for its low market value. Currently, great interest has also been aroused by some crops, such as sugary sorghum and triticale, also with a view to crop rotation, which allows greater management flexibility and enhances the availability of substrates throughout the year (Cencič et al., 2007).

Sorghum is a promising alternative in the bioenergy sector, thanks to its agronomic characteristics and adaptability to limiting soil and climatic conditions. Compared to eucalyptus, for example, the production of sorghum dry matter is 40 t ha^{-1} in five months against the 20 t ha^{-1} in about seven years of the former (Pimentel et al., 2017). There are three agronomic types of sorghum: (i) biomass sorghum, whose crops have a high yield (about 30 t ha^{-1} of dry stem) and for this reason are more suitable for the generation of solid biofuels (i.e., direct combustion); (ii) forage sorghum, whose biomass has a lower lignin content in the stems than other crops and, therefore, is more suitable for the generation of liquid biofuels; and (iii) sugar sorghum, from which second-generation bioethanol is produced in yields similar to those of other biomass crops like sugar beet, maize, or sugar cane (Table 1.4). Therefore, sorghum has several potential uses that can be exploited by the bioenergy sector, according to the agronomic group and the part of the plant used (Pimentel et al., 2017).

The use of sorghum, when compared to other bioenergy crops, is advantageous both for its agronomic characteristics and for its adaptability to different pedo-climatic conditions. Compared to eucalyptus, for example, the production of sorghum dry matter is 40 t ha^{-1} in five months against the 20 t ha^{-1} in about seven years of the former. Pimentel et al. (2017) studied the productive potential of the three different sorghum agronomic types (biomass, sugar, and fodder) and concluded that in all the cultivars the production of biomass in the stalk (67-78 %) was greater than in the leaves (16-20 %) and panicle (3.6-11 %; Table 1.3). Only the panicles of the three types of cultivar showed acceptable moisture percentages (< 50 %; Vieira, 2012). The panicles also showed the highest hemicellulose content (biomass 37.4, sugar 39.8, forage 41.5 %; Table 1.5), while cellulose was higher in the stalk (biomass 29.1, sugar 28.3, forage 25.6 %; Table 1.5) and leaves (biomass 25.2, sugar 21.0, forage 26.9 %; Table 1.5) than in the panicles (biomass 22.7, sugar 15.7, forage 13.3 %; Table 1.5).

For ethanol (first and second generation) and bioelectricity production, as well as direct combustion, only the stalk is used. The other parts (panicle and leaves) are used as agroindustrial waste, for animal feed production, or for production of energy through pyrolysis. By comparison, the production of cellulose from eucalyptus has high costs because of its high lignin content and because the forest cycle is about seven years. The use of sorghum is therefore competitive since the bagasse of sugar sorghum contains, on average, 30% cellulose, against 34% for wheat straw, 40% for sugar cane bagasse, 32% for maize cob, and 46% for *Eucalyptus globulus* wood (Silva, 2010). The amount of lignin, which has a more complex structure compared to cellulose and hemicellulose, can positively influence the thermal degradation of biomass. The component that has the highest percentage of lignin is the stalk of biomass sorghum (18.7%).

Table 1.4. Average yields of biodiesel, pure vegetable oil, bioethanol, and bio-ETBE, and the lower anaerobic biogasification potential (ABP), biomethane production (BMP), and higher heating value (HHV) of the principal energy crops.

Crops	**Biodiesel (t ha^{-1})**	**Pure Vegetable Oil (t ha^{-1})**	**Bio-Ethanol (L ha^{-1})**	**Bio-ETBE (t ha^{-1})**	**ABP (Nm^{3} t^{-1} DM)**	**BMP (Nm^{3} CH$_4$ ha^{-1})**	**HHV (MJ kg^{-1})**
Rapeseed	0.9 [a]	0.8 [a]	-	-	-	-	-
Sunflower	1.1 [a]	1.0 [a]	-	-	-	832–4695 [b,c]	-
Soy	0.6 [a]	0.5 [a]	-	-	-	-	-
Abyssinian mustard	1.0 [a]	0.9 [a]	-	-	-	-	-
Sugar beet roots	-	-	5000-6000 [d,e,f]	9.6 [a]	-	1954-6309 [c]	-
Sugar sorghum stems	-	-	2800-6000 [h,m,n,f]	8.3 [a]	423 [g]	2124–8370 [c,i,l]	-
Maize	-	-	grains 700-3232 [h,m,n,q] starch 2010-4000 [d,r] stover 700-2000 [q,r]	7.2 [a]	694 [g]	5453-7768 [o,p] 5300–9000 [b] 5862-12150 [b,c,i]	stover 18.4 [g]
Common cane	-	-	11000 [s]		524 [g]	9580-19,440 [t,u]	18.7 [g]
Triticale	-	-	2843 [v]		677 [g]	1000–5944 [c,i,z]	-
Miscanthus	-	-	8812 [q]		-	-	18.7 [g]
Sugar cane	-	-	3000-8000 [e,f,h,n,m]		-	-	16.8 [g]

[a] (Cencič et al., 2007); [b] (Amon et al., 2007); [c] (Murphy et al., 2011); [d] (Adams et al., 2009); [e] (Ziska et al., 2009); [f] (Almodares and Hadi, 2009); [g] (Corno et al., 2014); [h] (Jansson et al., 2009); [i] (Schievano et al., 2014); [l] (Seppälä et al., 2013); [m] (Ravindranath et al., 2011); [n] (Duku et al., 2011); [o] (Thyø and Wenzel, 2007); [p] (Oslaj et al., 2010); [q] (Quin et al., 2011); [r] (Keshwani and Cheng, 2009); [s] (Williams et al., 2008); [t] (Ragaglini et al., 2014); [u] (Corno et al., 2014); [v] (average value from Rosenberger et al. (2002), Table 8); [z] (Gatta et al., 2013).

Forage sorghum cultivars have a low concentration of lignin in the stalk (5.75%; Table 1.3). This makes them good candidates for hydrolysis, which yields sugars for the production of second-generation ethanol and, consequently, for the production of liquid biofuels. The ash content ranges from 3.41% to 9.44% (Table 1.3), values close to those of the maize cob (1.1% DW) and sugar cane bagasse (11.3% FW; Demirbas, 2004). Plant ashes contain reasonable quantities of micro- and macro-nutrients and are effective for reducing soil acidity, increasing the pH of the soil and its soluble concentrations of Mg, P, and K, and, therefore, can be used as fertilizers. However, in the bioenergy industries that use pyrolysis, the production of a great amount of ashes represents a disadvantage due to the need for their continuous removal from the furnace.

Arundo donax, or common cane, is an herbaceous perennial plant with a long, hollow, and robust stem that grows even in relatively poor soils. From its area of origin in the Mediterranean Basin and the Middle East, its distribution has expanded to temperate and subtropical regions of both hemispheres. It is a hydrophytic plant capable of growing in soils rich in water. Studies have shown that hereditary phenotypic differences exist among the clones of *A. donax* as regards, for example, the number, diameter, and height of the culms (Consentino et al., 2006a; Pilu et al., 2014). Therefore, the genetic improvement of the species could lead to an improvement in the production of biomass. The use of *A. donax* for the production of bioenergy is justified not only by its high biomass yields (37.7 t ha^{-1}; Table 1.3) but also because it has proven application in the production of bioenergy/biofuels, both through AD for the production of biogas and through direct combustion of its biomass (Table 1.4). Its anaerobic biogasification potential (ABP; 524 Nm^3 t^{-1} DM) is about 75%, 77%, and 124% of those of maize (694 Nm^3 t^{-1} DM), triticale (677 Nm^3 t^{-1} DM), and sorghum (423 Nm^3 t^{-1} DM), respectively, in terms of productivity per unit area (40 t ha^{-1}). The average production of biomethane from these plants (12618 ± 3588 Nm^3 t^{-1} DM) varies according to the agronomic environment but is still higher than that of traditional energy crops (Table 1.4).

Table 1.5. Cellulose, hemicellulose, and lignin contents of principal energy crops.

Crops	Part of the plant	Cellulose (%)	Lignin (%)	Hemicellulose (%)	Reference
Biomass sorghum	Panicle	22.73	6.91	37.37	Pimentel et al. (2017)
	Leaf	25.22	5.51	34.76	
	Stalk	29.14	18.69	28.77	
Sugar sorghum	Panicle	15.65	4.98	39.81	Pimentel et al. (2017)
	Leaf	20.98	4.82	34.03	
	Stalk	28.33	8.28	27.20	
	Bagasse	31.67	9.32	29.30	
Forage sorghum	Panicle	13.32	5.77	41.52	Pimentel et al. (2017)
	Leaf	26.90	6.28	34.30	
	Stalk	25.56	5.75	32.25	
Common cane	Leaves	31.1	16.8	28.8	Cosentino et al. (2007)
	Stems	32.7		28.9	
	Bagasse	40			Pimentel et al. (2017)
Corn	Plant	21.9-19.9	1.4-2.1	20.8-17.3	Giunco et al. (2015)
	Panicle	32			Pimentel et al. (2017)
Miscanthus	Leaves	41.2		24.2	Cosentino et al. (2007)
	Stems	41.3	12.8	28.6	
Cardoon	Plant	41.4	7.3	23.6	Palumbo and Mastro (2014)
Abyssinian mustard	Plant	5.26	1.90	4.94	Xin et al. (2013)
Sunflower	Seeds	43.28		2.37	Calabrò (2018)
Soy	Soybean bark	45.9	2.3	18	
Sugar beet	Pulp	13.18	3.82	17	
Triticale	Plant	35.1	5.1	19.4	
Switchgrass	Plant	31.0	17.6	20.4	Palumbo and Mastro (2014)

With regard to the production of bioethanol, *A. donax* produces about 10,000 L ha^{-1}, at least 50% more than sugar cane (3000–6756 L ha^{-1}) and sugar beet (5000–6000 L ha^{-1}), and about 20% more than Miscanthus (8812 L ha^{-1}). The use of *A. donax* for direct combustion is promising as its higher heating value (HHV, 18.7 MJ kg^{-1}) is higher than or not significantly different from those of other crops such as sugar cane (16.8 MJ kg^{-1}), maize (18.4 MJ kg^{-1}), Miscanthus (18.7 MJ kg^{-1}), switchgrass (19.1 MJ kg^{-1}), and poplar (19.5 MJ kg^{-1}; Corno et al., 2014). However, the high ash production (11.3 t ha^{-1}) can be a problem for combustion in comparison to other crops (Table 1.3). Monti et al. (2008) highlighted that the part of the plant that produces more ash is the leaves and, therefore, with proper agronomic management, this problem could be overcome. The synthesis of both biomethane and bioethanol yields reusable secondary products for the production of more bioenergy and/or bioproducts. For example, AD yields, as a secondary product, a digestate, usable as organic fertilizer (Ledda et al., 2013). In this way, the sustainability of the total energy production process is improved. Studies carried out by Corno et al. (2016) on AD showed that *A. donax* was able to effectively replace maize by reducing the production costs of both biomass and electricity.

5. CULTIVATION TECHNIQUES

Biomass sorghum (*Sorghum bicolor* L.) has recently become a crop of extreme interest for bioenergy production because of the high yields that it can reach and the relatively simple cultivation technique that it requires. This species belongs to the family Poaceae, like grain crops and sugar cane, and is suitable for cultivation in temperate zones, with a spring-summer cycle. From the morphological point of view, between biomass and sugar sorghum there are no substantial differences. However, in terms of composition, the two varieties are quite different, and this justifies their different uses (Gelleti et al., 2006). In the fiber type, the production of structural carbohydrates, and in particular cellulose, prevails; whereas in the sugar type, at least 30% of all the accumulated dry matter consists of simple sugars (sucrose, glucose, and fructose). Sorghum is native to central–eastern Africa (Sudan, Ethiopia), but it is currently widespread in Africa and the USA, although still little diffused in Europe. It has a minimum temperature for germination of 13 °C and an optimal growth temperature range of 24–25 °C. It prefers medium-textured soils and is moderately tolerant of salinity. Fiber (biomass) sorghum is a variety characterized by high efficiency in the use of water and can be grown in semi-arid and Mediterranean environments in conditions of limited water supply. Experimental tests have shown some variability in biomass yield, from 15 to over 40 t ha^{-1} of dry matter (Bonari et al., 2004). In the Mediterranean area, medium–late-cycle hybrids (emergence-flowering period of more than 90 days) are preferred as they provide maximum yield with a smaller amount of grain at the time of collection. The harvest can be carried out 10-20 days before the flowering phase - when the accumulation of dry matter and cellulose in the culm is highest (Gelleti et al., 2006).

Sugar sorghum (*Sorghum bicolor* L. var. saccharatum) belongs to the Poaceae family. The composition of the plant diverges considerably, as mentioned above, from the fiber types. The marrow in sugar types is rich in sucrose (15%–20%), but the presence of free glucose and fructose hinders the crystallization of sucrose, making it technically impossible to use sugar sorghum for the production of crystallized sugar. Sugar sorghum is usually used as fodder or for the production of molasses (Gelleti et al., 2006). The pedoclimatic requirements and the main cultivation techniques are very similar to those of fiber sorghum. The high content of sugars and the high water content of the plant stems cause major problems for the storage of the collected biomass (e.g., fermentation). The fresh matter yield is around 80 t ha^{-1} (Gelleti et al., 2006). Bio-ethanol, bio-ETBE, biogas, and biomethane can be obtained from this plant in yields similar to those of other common energy crops (Table 1.4).

Common cane (*Arundo donax* L.), also known as common reed, is a perennial species of the Poaceae and is typical of the Mediterranean regions. It can grow spontaneously and is cultivated in small areas, mainly for the production of supports for vines or vegetable crops (Corno et al., 2014). It grows evergreen in different environments, but it is sensitive to low temperatures, which can also compromise the vitality of the rhizomes when they grow near the surface (Gelleti et al., 2006). This species is not an aquatic plant and does not particularly like stagnant water. Regarding soil, it is widely adaptable, although it prefers deep, sandy soils that are rich in organic matter, alkaline, and rich in calcium. Clay, shallow, and impermeable soils are not appropriate for this species. Yields exceeding 40 t ha^{-1} of dry matter can be obtained from the second cropping year (Gelleti et al., 2006).

Propagation and implantation represent two aspects of the agronomic techniques that have not yet been optimized and which greatly influence the economics of the crop. Some studies are in progress to identify suitable solutions for the collection of rhizomes (Corno et al., 2014). Lately, techniques that use plants obtained through in vitro micro-propagation have been increasingly gaining ground as they ensure uniformity of growth, high production standards, the possibility of mechanizing operations, and practically 100% rooting. Since domestic cane is a plant with a high photosynthetic capacity and is able to make the best use of soil fertility, soil nutrition can be easily managed - for example, using sewage sludge and/or the digestate that is normally available from the biogas producers (Gelleti et al., 2006). Bio-ethanol and BMP yields are particularly high for this species when compared to other energy crops (Table 1.4), which consequently shows high energy yields and CO_2-saving potential (Table 1.6).

Miscanthus refers to the species *Miscanthus sinensis* Anderss, one of 14–20 species within the genus *Miscanthus* (part of the Poaceae family). These plants are native to Southeast Asia and were initially introduced into Europe in the 20th century as ornamental plants. Miscanthus has adapted well to temperate climates and is resistant to low temperatures. Although the shoots and leaves can be damaged at temperatures close to 0 °C, the rhizomes, if well developed, can remain viable at temperatures below 0 °C for a long time (Gelleti et al., 2006). Regarding soil preferences, although widely adaptable, Miscanthus plants prefer deep, sandy soils rich in organic matter. The biomass of Miscanthus benefits greatly from a supply of water and, therefore, fully develops its production potential in well-irrigated areas (Gelleti et al., 2006). The interest in this species is relatively recent and, consequently, there is only a limited number of studies on the genetic variability, for both morphological and productive characters. Currently, the most used genotype in experimental sites is *Miscanthus x giganteus*, a sterile Miscanthus hybrid of *M. sinensis* and *M. sacchariflorus*. The different multi-year trials carried out with this genotype indicate that the average yields in the period of maximum accumulation of dry matter are equal to about 30 t ha^{-1} $year^{-1}$ from the year subsequent to that of establishment. The “giganteus” variety can only be propagated by vegetative means, using rhizomes or micro-propagated seedlings. Multiplication by rhizomes can be achieved through mechanical extirpation from one-year-old plants: the stumps can be fragmented into rhizomes of acceptable size by a rotary tiller, while commercially available machines, suitably modified, can be used for the collection of rhizomes from the soil (Di Candilo and Facciotto, 2012). This species has an elevated bio-ethanol and bio-ETBE yield (Table 1.4) and a relatively low LHV (Table 1.6)

Table 1.6. Lower heating value (LHV), energy yield (difference between the energy content of the biomass produced and the auxiliary energy fed into the cropping system), yield (ratio of output and energy inputs), and CO_2 saving of principal energy crops.

Crops	**LHV ($MJ\ kg^{-1}$)**	**Energy Yield ($GJ\ ha^{-1}$)**	**Yield**	**CO_2 Saving ($t\ CO_2\ eq.\ ha^{-1}\ year^{-1}$)**	**References**
Common cane	16.7-18.3	280 first year 592 from the second year	7.4 first year 77 from the second year	37.7	(Bracco et al., 2008)
Cardoon	14–17	133–344	7–31	19	(Bracco et al., 2008)
Abyssinian mustard	13 straw 20 panicle	4–44	1.7–13.4	0.2–2.4	(Bracco et al., 2008)
Sorghum	17.99	762 [a]	-	-	(Monti et al., 2006)
Maize	16.7	359 [a]	-	-	(Monti et al., 2006)
Miscanthus	11.92	179–378 [a]	-	-	(Monti et al., 2006)
Switchgrass	18.2	182–455 [a]	-	-	(Monti et al., 2006)

[a] Estimated using dry matter (Table 1.3) and LHV.

Switchgrass (*Panicum virgatum* L.) belongs to the family Poaceae. It is an herbaceous plant, C4, rhizomatous, perennial, and native to North America, where it has spread from the 55° N latitude down to Mexico as an important species in the prairies and pastures. It is used for many different purposes, including the production of energy (Petrini et al., 2005). Varieties from America can adapt to the climatic conditions of the Mediterranean area. Switchgrass is an undemanding crop, adapted to resist water stress and long periods of intense cold. Regarding the characteristics of the soil, its growth is generally favored in moist and loamy soils. The main processing of the soil and subsequent preparatory work do not differ from those normally applied for other renewal crops sown in late spring, such as maize and sorghum (Gelleti et al., 2006). Switchgrass requires an optimal seedbed preparation in order to achieve good seedling emergence. In the first year of cultivation, dry matter production levels of 6 to 12 t ha^{-1} are expected, while in the following years, from 14 to 24 t ha^{-1} of dry matter can be obtained (Gelleti et al., 2006). A relatively high LHV has been reported for this species (Table 1.6).

Sunflower (*Helianthus annuus* L.) belongs to the family Asteraceae (Compositae) and is the most important among the 100 species of the genus *Helianthus*. Sunflower is a crop with a spring–summer cycle that shows good adaptability and does not have an excessive demand for soil macronutrients. Being originally from environments with a temperate climate, it prefers relatively high temperatures (Gelleti et al., 2006). Sunflower plants do not grow well in loose substrates, as they tend to be sterile and unable to retain water, and prefer medium-textured, deep, or organic soils. This species is moderately tolerant of salinity. Although its root system has a tapering structure, sunflower does not possess a strong penetrative capacity in the soil and, therefore, it needs adequate preparatory interventions, especially in the most challenging soils. Sunflower, due to the rapidity of its development, normally has a suffocating effect on weeds. It must, however, be protected in the initial phases of the growing cycle (Brunetti et al., 2009). Biodiesel, pure vegetable oil and biogas/biomethane can be obtained from this species (Table 1.4).

Rapeseed (*Brassica napus* L.) belongs to the group of oleaginous plants, and in particular, to the family Brassicaceae (Cruciferae) and the genus *Brassica*. Spontaneous in Europe and in the Northwest of Africa, it is believed to have been domesticated after the nutritional value of the seeds of this weed, often infesting fields of cereals, was discovered. In the Mediterranean climate, the biological cycle of rapeseed is autumn-spring (Brunetti et al., 2009). Rapeseed is a microthermic plant and, therefore, does not need high temperatures to develop. This crop does not resist drought periods well, especially during the stages of rising and flowering. Rapeseed prefers temperate climates, humid and not too sunny. The crop is not particularly demanding: it prefers deep, fresh, fertile, and light soils, but it adapts to clay, calcareous, and peat ones, provided they are well drained. It shows moderate tolerance of salinity and low soil pH. For instance, in the dry regions of central and northern Italy, rapeseed can alternate with wheat. Particular attention must be paid to the preparation of the seedbed as the seeds are small. At the end of the growing cycle, the crop helps to enrich the soil with organic matter and nutritive elements derived from the abundant mass of its residues. As this species performs a good part of its cycle in cold and rainy months, it actively explores the soil, intercepting and taking up nitrate, thus helping to limit the risk of leaching (Gelleti et al., 2006). The average yields in central Europe (France–Germany) vary from 3 to 4 t ha^{-1} (Brunetti et al., 2009). The energy yield depends very much on the cultivation techniques and conditions and can vary between 4 and 44 GJ ha^{-1} (Table 1.6). In terms of CO_2 emissions, the savings are much lower for liquid biofuels than for solid ones; for rapeseed, they are estimated to be 0.2–2.4 t ha^{-1} year^{-1} in CO_2 equivalents (Consentino et al., 2005).

Sugar beet (*Beta vulgaris* L.) is cultivated for the production of sugar. It has been exploited with this aim since 1700, allowing sugar, once produced only from sugar cane, to become a product for general use. The sugar produced is sucrose, a disaccharide composed of glucose and fructose. The cultivation of sugar beet particularly benefits from deep and permeable soils that are well drained. The plant has good tolerance of saline soils. The preparation of the soil for sugar beet cultivation is done following normal agricultural practices, according to the type of soil: the tap-root must be able to penetrate deep in the soil and find no (hard) layers that limit its development, while waterlogging should be avoided (Giandon et al., 2010). The yield of tap-roots varies between 45 and 50 t ha^{-1} (Gelleti et al., 2006). Bio-ethanol, bio-ETBE and biogas can be obtained from this species in elevated yields (Table 1.4).

Maize (*Zea mays* L.) is a species native to the tropical and subtropical climate zones of the American continent. Introduced into Spain in 1493, maize spread quickly in other European countries, first for ornamental purposes, and a century later as a food resource. From Europe, mainly by the Portuguese, maize was spread in Africa and Asia, while it was taken to North America by the British. Maize is a plant with a summer cycle and is sown in spring; it is sensitive to cold, especially during the early stages of development (Gelleti et al., 2006). Regarding soil requirements, deep soils with pH between 6 and 6.5 are excellent for maize cultivation, which has high water requirements. Maize plants are large and have a root system that reaches to a depth of 1.5-2 m. Traditional soil preparation is based on plowing to 25 cm for loose soils and to 30-35 cm in heavy soils. Water availability is often one of the limiting factors, and about 300-400 m^3 ha^{-1} of water has to be supplied for irrigation. The dry matter yield varies from 22 to 26 t ha^{-1}. The grain is harvested with a moisture content of 21%-28%, usually 10–15 days after maturation (Giandon et al., 2010). Different bio-ethanol and biogas yields can be obtained when different parts of the plants are used for biofuel production (Table 1.4).

Cardoon (*Cynara cardunculus* L.), also known as the artichoke thistle, is a traditional horticultural multi-year crop characterized by high rusticity, being widespread throughout southern Europe. The cultivation is simple, as the cardoon can be propagated by seeds and normally does not need irrigation. In terms of productivity, the results obtained from experiments demonstrate that for low-input cultivation regimes, the biomass yields vary between 14 and 20 t ha^{-1} of dry matter (Consentino et al., 2005). With regard to oil production, taking into account the average results obtained from various studies carried out in Sicily and in Spain, a seed yield ranging from 0.4 to 2.8 t ha^{-1} is expected, with an oleic content that varies between 18% and 27%. The achenes (the achene represents the typical fruit of the Asteraceae, the botanical family to which the genus *Cynara* belongs), where the seeds are contained, represent, in total, 30% of the dry matter of the flower heads and about 8%–10% of the total biomass (Fernández and Curt, 2004; Foti et al., 1999; Gherbin et al., 2001; Piscioneri et al., 2000). It should be noted that in the cultivation of cardoon, both for biomass and for seed, yields decrease with time and tend to decrease more or less significantly from the third year onwards (Consentino et al., 2006b). From an energetic point of view, the biomass of the cardoon has an LHV equal to 14–17 MJ kg^{-1} (Table 1.6), with a considerable ash content (10%–16%) that increases the risk of slagging and fouling, even if the overall silica content is less of a problem and the melting temperature exceeds 1200 °C (Angelini et al., 1999). The average energy yield fluctuates around 133–344 GJ ha^{-1}, influenced by different crop factors. As far as carbon emissions are concerned (Table 1.6), cardoon can save about 19 t ha^{-1} per year of CO_2 eq (Consentino et al., 2006b).

Abyssinian mustard (*Brassica carinata* A. Braun) has an annual crop cycle and is mainly destined to the production of oil for biodiesel as it is not intended to be used as a food or fodder crop. It has been studied since the 1990s, together with other species of the

Brassicaceae, for its content of seed oil rich in long-chain fatty acids, which is of interest to the lipochemical industry. These studies have shown the excellent adaptability of this crop to southern European environments, in relation to the early flowering, the non-dehiscence of the siliqua (the typical fruit of the botanical Brassicaceae family), the resistance to lodging and the main biotic adversities, and the adaptability to the typical rainfed agriculture conditions (Bracco et al., 2008). In rotations, *B. carinata* can follow both cereals and legumes, while the cultivation of successive crops of *B. carinata* is not recommended due to the possible establishment of plant pathogens. In relation to yields, the available data show an average production of about 1.4 t ha^{-1}, with minimum and maximum values of 0.5 and 3.5 t ha^{-1}, respectively (Bracco et al., 2008; Mazzoncini and Angelini, 2002; Messina and Pecorino, 2008). The average seed moisture content at harvest is 5%, with an oil content of 43% and a total yield of about 1.44 t ha^{-1}. From an energetic point of view, this species has an HHV of about 40 MJ kg^{-1}, with low viscosity values, which makes it particularly suitable for the needs of the main engine manufacturers and better in comparison to the most common raw vegetable oils (Messina, 2008). However, this species shows moderate energy yield and CO_2 saving values (Table 1.6).

It can be therefore summarized that biomass sorghum has characteristics that allow its use in direct combustion and in energy cogeneration. The different parts of the plant can be used differently depending on their chemical composition and their percentage presence in the total biomass. Common cane, despite the variability in biomass production, produces much more biomass than other energy crops, leading to a much higher fuel/energy production per unit area (592 GJ ha^{-1} from the second year; Table 1.6). The data collected here suggest the use of this crop as a substitute for traditional energy crops, which would also reduce the costs of biomass production. Indeed, large-scale approaches confirm previous findings that the use of common cane was able to reduce both the costs of producing biogas and the total area needed to produce the energy crop, thus making biogas production more sustainable. This plant species can produce about 160 m^3 of biogas per tonne, but considering the high biomass production, one hectare of common cane produces a similar amount of biogas to 1.5 hectares of maize, but with a lower use of resources. The calorific value is comparable to that of other crops used for this purpose, but also in this case, the high productivity per hectare makes it very competitive, allowing energy performances above 200,000 kWh ha^{-1} (twice as much as other herbaceous crops and trees). This plant species could replace maize as a bioenergy crop, reducing the production costs of both biomass and electricity, due to either the higher biomass productivity or the reduction in the cost of the biomass. However, the colonizing capacity of common cane, due to its subterranean organs, could compromise the conditions of habitability of the soil (for the subsequent crop) at the end of the cropping cycle, which is why many agricultural producers are reluctant to cultivate it. For this reason, at the end of the cycle, it is necessary to implement a reclamation program consisting of a series of alternating cultivation operations, involving deep processes and chemical weeding before soil preparation for the next crop (Bracco et al., 2008). Cardoon, like common cane, is an endemic multi-year herbaceous species characterized by high productivity, and it is particularly adaptable to Mediterranean environments. Moreover, due to its low water and nutritional requirements, it is possible to hypothesize its cultivation in low energy-input systems. In fact, apart from the operations of planting, the interventions in the cultivation cycle are limited to the fertilization and collection of biomass. The use of cardoon as a bioenergy crop allows for more scenarios consisting of the exclusive production of biomass or the double production of biomass together with seeds for oil extraction (Encinar et al., 2002; Fernández and Curt, 2004; Foti et al., 1999). The low quantity of moisture present in the harvested biomass enhances its commercial quality for combustion. Despite the higher oil content of rapeseed (*B. napus*) (Messina and Pecorino, 2008), the cultivation characteristics of *B. carinata* give it greater production stability compared to rapeseed. Moreover, in the case of *B. carinata*, an advanced phase of experimentation is underway to confirm its adaptability to the hot, arid environment of Mediterranean arable land and to cultivation regimes with reduced use of technical means, as well as its suitability for inclusion in rotation programs with other food and fodder plants.

6. CONCLUSIONS AND PERSPECTIVES

In order to address the increasing demand for energy production and at the same time deal with environmental protection issues, the European Union has set the goal of increasing the share of renewable energy to 20%, among other objectives of the “Europe 2020 Strategy”. The studies carried out to date show that some plant species have particular physical and chemical characteristics for their use as biomass crops for bioenergy production, with clear advantages both in terms of energy production and at the economic level. Moreover, it is clear that the different techniques used to obtain bioenergy require different characteristics in plant biomass. For example, in direct combustion, the biomass at harvest should have low water (<10%-12%) and ash content and a high amount of lignin. In the production of ethanol or in gasification systems, the lignocellulosic components (such as cellulose and hemicellulose) are important. The most suitable materials for the production of biogas through the process of AD are those with a high content of organic matter; for example, the biomasses obtained from energy crops such as maize.

The use of plant biomass for the production of (bio)energy can therefore be considered a suitable and environmentally friendly option to achieve the desired renewable energy share. The appearance of new and alternative plant species, like the ones described in this review, may help to pave the way to the implementation of the different energy production alternatives that are currently being established and or developed, particularly in southern European countries. The lower fertilization needs and higher adaptability to different soil types, including degraded and contaminated ones, of some of these species compared to conventional crops used for bioenergy production, together with their elevated energy yield potential, make the cultivation of these plants both economically and environmentally profitable. In addition, the competition for land use and agricultural resources with food crops would be significantly diminished, a relevant step towards the necessary sustainability of agroecosystems in southern Europe.

REFERENCES

Abouelenien, F.; Namba, Y.; Kosseva, M.R.; Nishio, N.; Nakashimada, Y. Enhancement of methane production from co-digestion of chicken manure with agricultural wastes. *Bioresour. Technol.* **2014**, *159*, 80–87, doi:10.1016/j.biortech.2014.02.050.

Adams, J.M.; Gallagher, J.A.; Donnison, I.S. Fermentation study on *Saccharina latissima* for bioethanol production considering variable pre-treatment. *J. Appl. Phycol.* **2009**, *21*, 569–574, doi:10.1007/s10811-008-9384-7.

Adnan, A.I.; Ong, M.Y.; Nomanbhay, S.; Chew, K.W.; Show, P.L. Technologies for biogas upgrading to biomethane: A review. *Bioengineering* **2019**, *6*, 92, doi:10.3390/bioengineering6040092.

Alburquerque, J.A.; de La Fuente, C.; Ferrer Costa, A.; Carrasco, L.; Cegarra, J.; Abad, M.; Bernal, M.P. Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. *Biomass Bioen.* **2012**, *40*, 181–189, doi:10.1016/j.biombioe.2012.02.018.

Alkoaik, F.; Abdel-Ghany, A.; Rashwan, M.; Fulleros, R.; Ibrahim, M. Energy analysis of a rotary drum bioreactor for composting tomato plant residues. *Energies* **2018**, *11*, 449, doi:10.3390/en11020449.

Almodares, A.; Hadi, M.R. Production of bioethanol from sweet sorghum: A review. *Afr. J. Agric. Res.* **2009**, *4*, 772–780.

Amon, T.; Amon, B.; Kryvoruchko, V.; Machmüller, A.; Hopfner-Sixt, K.; Bodiroza, V.; Hrbek, R.; Friedel, J.; Pötsch, E.; Wagentristl, H.; et al. Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. *Bioresour. Technol*. **2007**, *98*, 3204–3212, doi:10.1016/j.biortech.2006.07.007.

Anaborapi, 2001. Gli alimenti e i Loro Valori Nutrizionali. Available online: http://www.anaborapi.it/index.php?option=com_content&view=article&id=125%3Aallevamento-manza&catid=29%3 Atecniche-allevamento&Itemid=31&limitstart=9 (accessed on 15 February 2018).

Angelidaki, I.; Alves, M.; Bolzonella, D.; Borzacconi, L.; Campos, J.L.; Guwy, A.J.; Kalyuzhnyi, S.; Jenicek, P.; Van Lier, J.B. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. *Water Sci. Technol*. **2009**, *59*, 927–934, doi:10.2166/wst.2009.040.

Angelidaki, I.; Karakashev, D.; Batstone, D.J.; Plugge, C.M.; Stams, A.J.M. Biomethanation and its potential. *Methods Enzymol.* **2011**, *494*, 327–351, doi:10.1016/B978-0-12-385112-3.00016-0.

Angelini, L.; Ceccarini, L.; Bonari, E. Resa, composizione chimica e valutazione energetica della biomassa di specie erbacee annuali per la produzione di energia. In Proceedings of the Atti XXXIII Convegno Annuale Società Italiana di Agronomia “Le colture non alimentari”, Legnaro (Pd), Italy, 20–23 September 1999; pp. 84–85.

Bernal, M.P.; Sommer, S.G.; Chadwick, D.; Qing, C.; Guoxue, L.; Michel, F.C. Current approaches and future trends in compost quality criteria for agronomic, environmental, and human health benefits. In *Advances in Agronomy*; Academic Press Inc.: Cambridge, MA, USA, 2017; Volume 144, pp. 143–233, doi:10.1016/bs.agron.2017.03.002.

Bonari, E.; Picchi, G.; Guidi, W.; Piccioni, E.; Fraga, A.; Villani, R.; Ginanni, M. Le colture da energia. In: *Le Colture Dedicate ad Uso Energetico: Il Progetto Bioenergy Farm*, 1st ed.; Arsia: Firenze, Italy, 2004; Quaderno 6, pp. 29–78.

Bracco, S.; Chinnici, G.; Longhitano, D.; Santeramo, F.G. Prime valutazioni sull’impatto delle produzioni agroenergetiche in Sicilia. In Proceedings of the XLV Convegno SIDEA Sessione parallela: “Bioenergie”, Portici, Italy, 25–27 September 2008.

Braun, R. Anaerobic digestion: A multi-faceted process for energy, environmental management and rural development. In *Improvement of Crop Plants for Industrial Uses*; Ranalli, P., Ed.; Springer: Dordrecht, The Netherlands, 2007; Chapter 13, pp. 335–416.

Brown, D.; Shi, J.; Li, Y. Comparison of solid-state to liquid anaerobic digestion of lignocellulosic feedstocks for biogas production. *Bioresour. Technol.* **2012**, *124*, 379–386, doi:10.1016/j.biortech.2012.08.051.

Brunetti, G.; Casulli, G.; Cazzato, E.; De Mastro, G.; Disciglio, G.; Fracchiolla, M.; Grassano, N.; Pinto, F.; Tarantino, E.; Tedone, L.; et al. Girasole (*Helianthus annus L.*). In *Quaderno Colture Erbacee*, 1st ed.; Piepoli, M.G., Cardone, A., Antonicelli, M., Bulzis, P.S., De Castro, F., Savino, V.N., Eds; Centro di Ricerca e sperimentazione in Agricoltura “Basile Caramia”: Bari, Italy, 2009; pp. 165–178.

Busch, O.; Schade, A.; Rasch, H.; Schulte-Koerne, E. Bio-MTBE. How to reduce CO_2 footprint in fuels with a well known premium gasoline component. In Proceedings of the Conference: ‘‘Reducing the Carbon Footprint of Fuels and Petrochemicals’’, Berlin, Germany, 8–10 October 2012.

Calabrò, S. (2018) Valutazione Nutrizionale Degli Alimenti e Dei Sottoprotti. Available online: http://www.federica.unina.it/medicina-veterinaria/valutazione-nutrizionale-degli-alimenti-e-dei-sottoprodotti/valutazione-sottoprodotti/ (accessed on 22 February 2018).

Candolo, G. (2005) Biomasse Vegetali : I Possibili Processi di Conversione Energetica. Available online: http://www.crpa.it/media/documents/crpa_www/Progetti/Seq-Cure/Candolo1.pdf (accessed on 28 February 2018).

Cavinato, C.; Fatone, F.; Bolzonella, D.; Pavan, P. Thermophilic anaerobic co-digestion of cattle manure with agro-wastes and energy crops: Comparison of pilot and full scale experiences. *Bioresour. Technol.* **2010**, *101*, 545–550, doi:10.1016/j.biortech.2009.08.043.

Cencič, V.; Jodice, R.; Migliardi, D.; Picco, D.; Pin, M.; Tomasinsig, E.; Zanotto, G.; Radu, R.; Taccani, R.; Chindaruksa, S.; Barbieri, S., Bianco, D., Michelutti, R., Monaco, B. *I Biocarburanti. Le Filiere Produttive, le Tecnologie, i Vantaggi Ambientali e le Prospettive di Diffusione*; Area Science Park: Padriciano, Italy, 2007; Volume 27, pp. 26-158. Copyright © 2007 by Consorzio per l’AREA di ricerca scientifica e tecnologica di Trieste AREA Science Park. Padriciano, 99 - 34012 Trieste.

Chiaramonti, D. Bioethanol: Role and production technologies. In *Improvement of Crop Plants for Industrial Uses*; Ranalli, P., Ed.; Springer: Dordrecht, The Netherlands, 2007; Chapter 8, pp. 209–252.

Ciaschini, F.; De Carolis, C.; Toscano, G. Technical and economic aspects of mechanical extraction of sunflower oil for energy purposes. Proceedings of National Conference of Agrarian engineering for sustainable development of the Mediterranean area, Catania, Italy, 27–30 June 2005.

Corno, L.; Lonati, S.; Riva, C.; Pilu, R.; Adani, F. Giant cane (*Arundo donax L.*) can substitute traditional energy crops in producing energy by anaerobic digestion, reducing surface area and costs: A full-scale approach. *Bioresour. Technol.* **2016**, *218*, 826–832. http://doi.org/10.1016/j.biortech.2016.07.050.

Corno, L.; Pilu, R.; Adani, F. *Arundo donax* L.: A non-food crop for bioenergy and bio-compound production. *Biotechnol. Adv.* **2014**, *32*, 1535–1549, doi:10.1016/j.biotechadv.2014.10.006.

Cosentino, S.; Foti, S.; Venturi, G.; Giovanardi, R.; Copani, V.; Mantineo, M.; D'Agosta, G.; Bezzi, G.; Tassan Mazzocco, G. *Colture Erbacee Annuali e Poliennali da Biomassa per Energia di Possibile Coltivazione in Italia*; Research Institute for Industrial Crops: Bologna, Italy, 2005; Volume 1, pp. 35–48.

Cosentino, S.L.; Bona, S.; Copani, V.; Patanè, C.; Mantineo, M.; D'agosta, G. (2006b) Aspetti agronomici, energetici ed ambientali di colture da biomassa per energia per gli ambienti italiani. In Proceedings of the L'agricoltura per l'Energia Rinnovabile: I Futuri Scenari, Potenza, Italy, 23–24 November 2006.

Cosentino, S.L.; Copani, V.; D'Agosta, G.M.; Sanzone, E.; Mantineo, M. First results on evaluation of *Arundo donax* L. clones collected in Southern Italy. *Ind. Crops Prod.* **2006a**, *23*, 212–222, doi:10.1016/j.indcrop.2005.06.004.

Cosentino, S.L.; Sanzone, E.; Bellomia, L.; Sortino, O.; Belligno, A. Chemical composition and energetic value of perennial ligno-cellulosic species (*Miscantus x Giganteus* Greef and Deu. and *Arundo donax* L.) grown in Mediterranean region. In Proceedings of the 15th European Biomass Conference & Exhibition, Berlin, Germany, 7–11 May 2007.

Cui, Z.; Shi, J.; Li, Y. Solid-state anaerobic digestion of spent wheat straw from horse stall. *Bioresour. Technol.* **2011**, *102*, 9432–9437, doi:10.1016/j.biortech.2011.07.062.

Demirbas, A. Biofuels. In *Biohydrogen. For future engine fuel demands*; Springer-Verlag: London, UK, 2009; pp. 61-84.

Demirbas, A. Combustion characteristics of different biomass fuels. *Prog. Energy Combust. Sci*. **2004**, *30*, 219–230, doi:10.1016/j.pecs.2003.10.004.

Deublein, D.; Steinhauser, A. *Biogas from Waste and Renewable Resources*; Wiley-VCH Verlag GmbH & Co KGaA: Weinheim, Germany, 2008.

Di Candilo, M.; Facciotto, G. Colture da biomassa ad uso energetico Potenzialità e prospettive. In *Progetti di ricerca SUSCACE e FAESI—Recenti acquisizioni scientifiche per le colture energetiche*; Pari, L., Ed.; Compagnia delle Foreste: Arezzo, Italy, 2012; Sherwood 183, Supplemento 2, pp. 10–19.

Duku, M.H.; Gu, S.; Hagan, E.B. A comprehensive review of biomass resources and biofuels potential in Ghana. *Renew. Sust. Energy Rev*. **2011**, *15*, 404–415, doi:10.1016/j.rser.2010.09.033.

EEA. *Trends and Projections in Europe 2015: Tracking Progress towards Europe's Climate and Energy Targets*; European Environment Agency: Copenhagen, Denmark, 2015; Report 4, pp. 16–55.

EEA. *Trends and Projections in Europe 2018: Tracking Progress towards Europe's climate and Energy Targets*; European Environment Agency: Copenhagen, Denmark, 2018; Report 16, pp. 16–55.

Encinar, J.M.; González, J.F.; Rodríguez, J.J.; Tejedor, A. Biodiesel fuels from vegetable oils: Transesterification of *Cynara cardunculus L.* oils with ethanol. *Energy Fuels* **2002**, *19*, 443–450, doi:10.1021/ef010174h.

Eurostat. *Archive: Energy from Renewable Sources*; Eurostat: Luxembourg, 2017; pp. 1–14. © European Union.

Fernández, J.; Curt, D. Low-cost biodiesel from *Cynara* oil. In Proceedings of the 2nd World Conference on Biomass for Energy, Rome, Italy, 10–14 May 2004.

FitzPatrick, M.; Champagne, P.; Cunningham, M.F.; Whitney, R.A. A biorefinery processing perspective: Treatment of lignocellulosic materials for the production of value-added products. *Bioresour. Technol.* **2010**, *101*, 8915–8922, doi:10.1016/j.biortech.2010.06.125.

Foti, S.; Mauromicale, G.; Raccuia, S.A.; Fallico, B.; Fanella, F.; Maccarone, E. Possible alternative utilization of *Cynara spp*.: I. Biomass, grain yield and chemical composition of grain. *Ind. Crops Prod.* **1999**, *10*, 219–228, doi:10.1016/S0926-6690(99)00026-6.

Gatta, G.; Gagliardi, A.; Soldo, P.; Monteleone, M. Grasses and legumes in mixture: An energy intercropping system intended for anaerobic digestion. *Ital. J. Agron.* **2013**, *8*, 47–57.

Gebreegziabher, T.; Oyedun, A.O.; Hui, C.W. Optimum biomass drying for combustion—A modeling approach. *Energy* **2013**, *53*, 67–73, doi:10.1016/j.energy.2013.03.004.

Gelleti, R.; Jodice, R.; Mauro, G.; Migliardi, D.; Picco, D.; Pin, M.; Tomasinsi, E.; Tommasoni, L.; Chinese, D.; Monaco, B.; et al. Allegato 1: Schede colturali delle principali specie a fini energetic. In *Energia delle Biomasse: Le Tecnologie, i Vantaggi per i Processi Produttivi, i Valori Economici e Ambientali*, 1st ed.; Area Science Park: Padriciano, Italy, 2006; Volume 24, pp. 187–214.

Gherbin, P.; Monteleone, M.; Tarantino, E. Five year evaluation on Cardoon (*Cynara cardunculus* L. var. altilis) biomass production in a Mediterranean Environment. *Ital. J. Agron.* **2001**, *5*, 11–19.

Giandon, P.; Talbot, S.; Obber, S.; Garlato, A.; Ferasin, M. Barbabietola da Zucchero (*Beta vulgaris*). In *Colture e Nergetiche e Protezione del Suolo;* ARPAV, Ed.; Veneto Agricoltura: Padova, Italy, 2010; p. 35.

Giovanardi, R.; Castelluccio, M.D.; Rosso, S.; Mazzocco, G.T.; Picco, D.; Agronomici, A.; Ambientali, E. *La Coltivazione del Miscanto in Friuli Venezia Giulia: Aspetti Agronomici, Energetici e Ambientali*; *Notiziario* ERSA: Gorizia, Friuli Venezia Giulia, Italy, 2009; 3, pp. 42–48.

Giuliano, A.; Bolzonella, D.; Pavan, P.; Cavinato, C.; Cecchi, F. Co-digestion of livestock effluents, energy crops and agro-waste: Feeding and process optimization in mesophilic and thermophilic conditions. *Bioresour. Technol.* **2013**, *128*, 612–618, doi:10.1016/j.biortech.2012.11.002.

Giunco, G. Valutazione Della Degradabilità e Della Produzione di Gas in vitro di Differenti Ibridi di Sorgo e di Mais Insilati Provenienti da Due Aziende Della Pianura Padana. Master’s Thesis, Department of Agronomy Animals Food Natural Resources and the Environment, University of Padova, Padua, Italy, 2015. Available online: http://tesi.cab.unipd.it/49484/1/Giunco_Giorgia.pdf (accessed on 22 February 2018).

Hu, Z.H.; Wang, G.; Yu, H.Q. Anaerobic degradation of cellulose by rumen microorganisms at various pH values. *Biochem. Eng. J.* **2004**, *21*, 59–62, doi:10.1016/j.bej.2004.05.004.

Hu, Z.H.; Yu, H.Q. Application of rumen microorganisms for enhanced anaerobic fermentation of corn stover. *Process Biochem.* **2005**, *40*, 2371–2377, doi:10.1016/j.procbio.2004.09.021.

Iaboni, V.; De Stefanis, P. La valorizzazione delle scorie di combustione dei rifiuti urbani. *Complessità Sviluppo*. **2003**, *1*, 146–159.

IEA. Sustainable production of second-geneation biofuels. In *Potential and Perspectives in Major Economies and Developing Countries*; IEA: Paris, France, 2010.

Jahirul, M.I.; Rasul, M.G.; Chowdhury, A.A.; Ashwath, N. Biofuels production through biomass pyrolysis-A technological review. *Energ.* **2012**, *5*, 4952–5001, doi:10.3390/en5124952.

Jansson, C.; Westerbergh, A.; Zhang, J.; Hu, X.; Sun, C. Cassava, a potential biofuel crop in (the) People's Republic of China. *Appl. Energy* **2009**, *86*, 95–99, doi:10.1016/j.apenergy.2009.05.011.

Kampman, B.; Leguijt, C.; Scholten, T.; Tallat-Kelpsaite, J.; Brückmann, R.; Maroulis, G.; Lesschen, J.P.; Meesters, K.; Sikirica, N.; Elbersen, B. *Optimal Use of Biogas from Waste Streams. An Assessment of the Potential of Biogas from Digestion in the EU beyond 2020*; European Commission: Brussels, Belgium, 2016.

Karthikeyan, O.P.; Visvanathan, C. Bio-energy recovery from high-solid organic substrates by dry anaerobic bio-conversion processes: A review. *Rev. Environ. Sci. Bio.* **2012**, *12*, 257–284. http://dx.doi.org/10.1007/s11157-012-9304-9.

Keshwani, D.R.; Cheng, J.J. Switchgrass for bioethanol and other value-added applications: A review. *Bioresour. Technol.* **2009**, *100*, 1515–1523, doi:10.1016/j.biortech.2008.09.035.

Khanal, S.K. *Anaerobic Biotechnology for Bioenergy Production: Principles and Applications*, 1st ed.; John Wiley & Sons Inc.: Ames, IA, USA, 2008.

Kotay, S.; Das, D. Biohydrogen as a renewable energy resource-prospects and potentials. *Int. J. Hydrogen Energ.* **2008**, *33*, 258–263, doi:10.1016/j.ijhydene.2007.07.031.

Ledda, C.; Schievano, A.; Salati, S.; Adanim, F. Nitrogen and water recovery from animal slurries by a new integrated ultrafiltration, reverse osmosis and cold stripping process: A case study. *Water Resour.* **2013**, *47*, 6157–6166, doi:10.1016/j.watres.2013.07.037.

Lehtomäki, A.; Viinikainen, T.A.; Rintala, J.A. Screening boreal energy crops and crop residues for methane biofuel production. *Biomass Bioenergy* **2008**, *32*, 541–550. https://dx.doi.org/10.1016/j.biombioe.2007.11.013.

Liew, L.N.; Shi, J.; Li, Y. Methane production from solid-state anaerobic digestion of lignocellulosic biomass. *Biomass Bioener.* **2012**, *46*, 125–132, doi:10.1016/j.biombioe.2012.09.014.

Mancini, A.M.; Berton, C.M.; Apote, C.L.D.; Pari, C.L.; Sandro, E.; Andrea, S.D. Caratteristiche tecniche delle biomasse e dei biocombustibili. In *Progetto Biomasse*; Enama: Rome, Italy, 2011; pp. 2–14.

Mantineo, M.; D'Agosta, G.M.; Copani, V.; Patanè, C.; Cosentino, S.L. Biomass yield and energy balance of three perennial crops for energy use in the semi-arid

Mediterranean environment. *Field Crops Res.* **2009**, *114*, 204–213, doi:10.1016/j.fcr.2009.07.020.

Marrone, N. *Le Prospettive di Crescita Delle Energie Rinnovabili in Puglia: Il Parco Delle Biomasse*, 1st ed.; FrancoAngeli: Milano, Italy, 2014; pp. 54–58.

Mazzoncini, M.; Angelini, L. Brassicaceae e nuove specie oleaginose per usi industriali non alimentari, *Ital. J. Agron.* **2002**, *36*, 53–68.

Messina, B. Risultati dei campi di moltiplicazione e delle prove sperimentali– Progetto della Regione Sicilia: Fi.Sic.A. "Filiera Siciliana per l'Agroenergia". In Proceedings of the Atti del Convegno, Raddusa, Italy, 3 July 2008.

Messina, B.; Pecorino, B. "*Filiera Siciliana per l'Agroenergia": Prime Valutazioni dei Costi di Produzione Della Brassica Carinata e Della Produzione di Biodiesel in un Esempio di Filiera Corta*; PROGETTO Fi.Sic.A.; Department of Economic-Agricultural and Estimative Sciences, University of Catania: Catania, Italy, 2008. Available online: http://www.ilgranoduro.it/osservatorio/rapporto4/15-parte-quindicesima.pdf (accessed on 15 February 2018).

Monti, A., Pritoni, G., Venturi, G., Canestrale, R. (2006) Colture da BIOMASSA : Panico e Canna Comune, Performance a Confronto. Available online: http://www.crpv.it/doc/5229/Canestrale.pdf (accessed on 10 December 2018).

Monti, A.; Di Virgilio, N.; Venturi, G. Mineral composition and ash content of six major energy crops. *Biomass Bioener.* **2008**, *32*, 216–223, doi:10.1016/j.biombioe.2007.09.012.

Murphy, J.; Bochmann, G.; Weiland, P.; Wellinger, A. Biogas from crop digestion. *IEA Bioenergy* **2011**, *Task 37*, 24.

Naik, S.N.; Vaibhav, V.G.; Prasant, K.R.; Ajay, K.D. Production of first and second generation biofuels: A comprehensive review. *Renew. Sust. Energ. Rev.* **2010**, *14*, 578–597, doi:10.1016/j.rser.2009.10.003. © 2009 Elsevier Ltd.

Neugebauer, M. Kitchen and garden waste as a source of heat for greenhouses. *Agric. Eng.* **2018**, *22*, 83–93, doi:10.1515/agriceng-2018-0008.

Nizami, A.S.; Korres, N.E.; Murphy, J.D. Review of the integrated process for the production of grass biomethane. *Environ. Sci. Technol.* **2009**, *43*, 8496–8508, doi:10.1021/es901533j.

Onwosi, C.O.; Igbokwe, V.C.; Odimba, J.N.; Eke, I.E.; Nwankwoala, M.O.; Iroh, I.N.; Ezeogu, L.I. Composting technology in waste stabilization: On the methods, challenges and future prospects. *J. Environ. Manag.* **2017**, *190*, 140–157, doi:10.1016/j.jenvman.2016.12.051.

Oslaj, M.; Mursec, B.; Vindis, P. Biogas production from maize hybrids. *Biomass Bioener.* **2010**, *34*, 1538–1545, doi:10.1016/j.biombioe.2010.04.016.

Palumbo, A.; Mastro, G. Produzione di "bioetanolo di seconda generazione" dal cardo (*Cynara cardunculus* L. var. altilis DC.). In Proceedings of the SIA—Società Italiana di Agronomia, XLIII Convegno Nazionale, Pisa, Italy, 17–19 September 2014.

Petersson, A.; Wellinger, A. Biogas Upgrading Technologies—Developments and Innovations. IEA Bioenergy 2009. Available online: www.iea-biogas.net (accessed on 02 May 2020).

Petrini, A.; Piccinini, M.; Fuselli, D.; Antonelli, M.; *Miglio*, 1st ed.; Scocco & Gabrielli: Tolentino, Italy, 2005; pp. 18–20.

Pilu, R.; Cassani, E.; Landoni, M.; Cerino Badone, F.; Passera, A.; Cantaluppi, E. Genetic characterization of Italian giant reed (*Arundo donax* L.) clones collection: Exploiting clonal selection. *Euphytica* **2014**, *196*, 169–181, doi:10.1007/s10681-013-1022-z.

Pimentel, L.D.; Batista, V.A.P.; de Barros, A.F.; Teófilo, R.F.; dos Santos Dias, L.A. Caracterização química e bioenergética de grupos agronômicos de sorgo. *Pesqui. Agropecu. Trop.* **2017**, *47*, 424–431.

Piscioneri, I.; Sharma, N.; Baviello, G.; Orlandini, S. Promising industrial energy crop, *Cynara cardunculus*: A potential source for biomass production and alternative energy. *Energy Convers. Manag.* **2000**, *41*, 1091–1105, doi:10.1016/S0196-8904(99)00135-1.

Plöchl, M.; Zacharias, H.; Herrmann, C.; Heiermann, M.; Prochnow, A. Influence of silage additives on methane yield and economic performance of selected feedstock. *E J. CIGR* **2009**, *11*, 1–16.

Protásio, T.d.P.; Bufalino, L.; Tonoli, G.H.D.; Guimarães Junior, M.; Trugilho, P.F.; Mendes, L.M. Brazilian lignocellulosic wastes for bioenergy production: Characterization and comparison with fossil fuels. *BioRes.* **2013**, *8*, 1166–1185.

Qin, Z.; Zhuang, Q.; Zhu, X.; Cai, X.; Zhang, X. Carbon consequences and agricultural implications of growing biofuels crops on marginal agricultural lands in China. *Environ. Sci. Technol.* **2011**, *45*,10765–72, doi:10.1021/es2024934.

Ragaglini, G.; Dragoni, F.; Simone, M.; Bonari, E. Suitability of giant reed (*Arundo donax*) for anaerobic digestion: Effect of harvest time and frequency on biomethane yield potential. *Bioresour. Technol.* **2014**, *152*, 107–115, doi:10.1016/j.biortech.2013.11.004.

Raposo, F.; De La Rubia, M.A.; Fernández-Cegrí, V.; Borja, R. Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures. *Renew. Sustain. Energ. Rev.* **2011**, *16*, 861–877. https://dx.doi.org/10.1016/j.rser.2011.09.008.

Ravindranath, N.H.; Lakshmi, C.S.; Manuvie, R.; Balachandra, P. Biofuel production and implications for land use, food production and environment in India. *Energy Policy* **2011**, *39*, 5737–5745, doi:10.1016/j.rser.2011.09.008.

Roos, C.J. *Biomass Drying and Dewatering for Clean Heat & Power*; WSU Extension Energy Program: Olympia, WA, USA, 2008.

Rosenberger, A.; Kaul, H.P.; Senn, T.; Aufhammer, W. Costs of bioethanol production from winter cereals: The effect of growing conditions and crop production intensity levels. *Ind. Crop Prod.* **2002**, *15*, 91–102. https://dx.doi.org/10.1016/j.rser.2011.09.008.

Rowe, R.L.; Steet, N.R.; Taylor, G. Identifying potential environmental impacts of large-scale deployment of dedicated bioenergy crops in the UK. *Renew. Sust. Energ. Rev.* **2009**, *13*, 271–290, doi:10.1016/j.rser.2007.07.008.

Saccani, C., Bianchini, A., Pellegrini, M. (2015) Biomasse. Available online: http://www.diem.ing.unibo.it/personale/saccani/index_files/Impianti%20Meccanici%20M%20(dal%202014-2015)/Impianti%20Meccanici%20M_modulo%201.1_Classificazione%20e%20caratterizzazione%20delle%20biomasse.pdf (accessed on 10 January 2018).

Santonoceto, C. Aspetti Nutrizionali Delle Oleaginose, Master's Thesis, Department of Agronomy, University of Reggio Calabria, Reggio Calabria, Italy, 2014.

Sawatdeenarunat, C.; Surendra, K.C.; Takara, D.; Oechsner, H.; Khanal, S.K. Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. *Bioresour. Technol.* **2015**, *178*, 178–186, doi:10.1016/j.biortech.2014.09.103.

Schievano, A.; D'Imporzano, G.; Orzi, V.; Colombo, G.; Maggiore, T.; Adani, F. Biogas from dedicated energy crops in Northern Italy: Electric energy generation costs. *GCB Bioenergy* **2014**, *7*, 899–908, doi:10.1111/gcbb.12186.

Seppälä, M.; Laine, A.; Rintala, J. Screening of novel plants for biogas production in northern conditions. *Bioresour. Technol.* **2013**, *139*, 355–362, doi:10.1016/j.biortech.2013.04.014.

Shammas, N.K.; Wang, L.K. Aerobic digestion. In *Biosolids Treatment Processes. Handbook of Environmental Engineering*; Wang, L.K., Shammas, N.K., Hung, Y.T., Eds.; Humana Press: Totowa, NJ, USA, 2007; Volume 6, pp. 177–205, doi:10.1007/978-1-59259-996-7_6.

Silva, N.L.C. Produção de Bioetanol de Segunda Geração a Partir de Biomassa Residual da Indústria de Celulose. Master's Thesis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2010.

Smith, M.M.; Aber, J.D. Energy recovery from commercial-scale composting as a novel waste management strategy. *Appl. Energy* **2018**, *211*, 194–199, doi:10.1016/j.apenergy.2017.11.006.

Smith, M.M.; Aber, J.D.; Rynk, R. Heat recovery from composting: A comprehensive review of system design, recovery rate, and utilization. *Compost Sci. Util.* **2017**, *25*, 11–22, doi:10.1080/1065657X.2016.1233082.

Takara, D.; Khanal, S.K. Green processing of tropical banagrass into biofuel and biobased products: An innovative biorefinery approach. *Bioresour. Technol.* **2011**, *102*, 1587–1592, doi:10.1016/j.biortech.2010.08.106.

Thyø, K.A.; Wenzel, H. *Life Cycle Assessment of Biogas from Maize Silage and from Manure—for Transport and for Heat and Power Production under Displacement of Natural Gas Based Heat Works and Marginal Electricity in Northern Germany*; Institut for Produktudvikling: Lyngby, Denmark, 2007.

Troussier, F., 2018. Lo Sviluppo del Biogas in Europa: Un Fututo Carico di Opportunità. Available online: https://rienergia.staffettaonline.com/articolo/33038/Lo+sviluppo+del+biogas+in+Europa:+un+futuro+carico+di+opportunit%C3%A0/Troussier (accessed on 4 September 2018).

Van Gerper, J. Biodiesel production. In *Improvement of Crop Plants for Industrial Uses*; Ranalli, P., Ed.; Springer: Dordrecht, The Netherlands, 2007; Chapter 10, pp. 281–290.

Vieira, A. C. (2012). Caracterização da biomassa proveniente de resíduos agrícolas. Dissertação (Mestrado em Energia na Agricultura) - Universidade Estadual do Oeste do Paraná, Cascavel. 72.

Vivarelli, S.; Tondi, G. Pyrolysis oil: An Innovative Liquid Biofuel for Heating. The COMBIO Project. In Proceedings of the International Workshop Bioenergy for a Sustainable Development, Casino Viña del Mar, Chile, 9 November 2004.

Wakker, A.; Egging, R.; van Thuijl, E.; van Tilburg, X.; Deurwaarder, E.; de Lange, T.; Berndes, G.; Hansson, J. *Biofuel and Bioenergy Implementation Scenarios*; Energy research Centre of the Netherlands: Petten, The Netherlands, 2005; Final Report of VIEWLS WP5, modelling studies (ECN-RX--05-141), p. 105.

Wallace, G.; Blondy, J.; Mirabella, W.; Oil, N. Ethyl tertiary butyl ether—A review of the technical literature. *SAE Int. J. Fuels Lubr.* **2009**, *2*, 940–952, doi:10.4271/2009-01-1951.

Williams, C.M.J.; Biswas, T.K.; Schrale, G.; Virtue, J.G.; Heading, S. Use of saline land and wastewater for growing a potential biofuel crop (*Arundo donax L.*). In Proceedings of the irrigation Australia Conference, Melbourne, Australia, 20–22 May 2008.

Wu, X.; Yao, W.; Zhu, J.; Miller, C. Biogas and methane productivity by co- digesting swine manure with three crop residues as an external carbon source. *Bioresour. Technol.* **2010**, *101*, 4042–4047, doi:10.1016/j.biortech.2010.01.052.

Xin, H.; Falk, K.C.; Yu, P. Studies on *Brassica carinata* seed. 2. Carbohydrate molecular structure in relation to carbohydrate chemical profile, energy values, and biodegradation characteristics. *J. Agric. Food Chem.* **2013**, *61*, 10127–10134, doi:10.1021/jf402077g.

Ye, J.; Li, D.; Sun, Y.; Wang, G.; Yuan, Z.; Zhen, F.; Wang, Y. Improved biogas production from rice straw by co-digestion with kitchen waste and pig manure. *Waste Manag.* **2013**, *33*, 2653–2658, doi:10.1016/j.wasman.2013.05.014.

Yusoff, M.N.A.; Zulkifli, N.W.M.; Masum, B.M.; Masjuki, H.H. Feasibility of bioethanol and biobutanol as transportation fuel in spark-ignition engine: A review. *RSC Adv.* **2015**, *5*, 100184–100211, doi:10.1039/C5RA12735A.

Zafar, S. (2018) Biomass Pyrolysis Process. Available online: http://www.bioenergyconsult.com/tag/flash-pyrolysis/ (accessed on 20 March 2018).

Ziska, L.H.; Runion, B.G.; Tomecek, M.; Prior, S.A.; Torbet, H.A.; Sicher, R. An evaluation of cassava, sweet potato and field corn as potential carbohydrate sources for bioethanol production in Alabama and Maryland. *Biomass Bioener.* **2009**, *33*, 1503–1508, doi:10.1016/j.biombioe.2009.07.014.

Zohri, A.A.; Ragab, S.W.; Mekawi, M.I.; Mostafa, O.A.A. Comparison between batch, fed-batch, semi-continuous and continuous techniques for bio-ethanol production from a mixture of Egyptian cane and beet molasses. *Egypt. Sugar J.* **2017**, *9*, 89–111.

CHAPTER 2. THE USE OF ENERGY CROPS FOR THE RECOVERY OF TRACE ELEMENTS CONTAMINATED SOILS

CHAPTER 2

THE USE OF ENERGY CROPS FOR THE RECOVERY OF TRACE ELEMENTS CONTAMINATED SOILS

1. INTRODUCTION

Soil contamination is a current major global problem due to the risk it presents for both the environmentand the public health. The areas subject to pollution and, therefore,that have to be reclaimed are numerous in Europe, and include fuel distribution and storage facilities, craft and industrial areas, mining areas, illegal landfills, waste disposal areas, etc. These areas are often a source of pollution for the surrounding environment due to the spread of pollutants through the dispersion of dust particles, lixiviation and soil erosion (Rodriguez-Eugenio et al., 2018). There are about three million potentially contaminated sites in Europe and more than 1,300 polluted or contaminated sites in the United States of America (Rodriguez-Eugenio et al., 2018). Despite there is no estimation of the actual extent of the pollution and its effects on the environment. Over 137,000 km^2 (6.24% of all agricultural soils in Europe) need remediation activities (Tóth et al., 2016).

However, the need of decontamining large areas is hampered by the high cost of currently available techniques, usually chemical engineering processes, which are also invasive and cause profound chemical, physical and biological changes in the soils subject to recovery. In fact, at the end of the remediation of the soil, this is often no longer suitable for cultivation because during the decontamination process all biological activity (useful microorganisms and soil fauna) is drastically impaired (Mancuso et al., 2004). To overcome these problems, research has focused on cheaper and more environmentally friendly alternative methods such as phytoremediation, defined as the use of plants to treat contaminated matrices (Salt et al., 1998; Raskin et al., 1999; Pulford et al., 2003; Barbafieri, 2005). The selection of the plant species is crucial for successful phytoremediation, as the plants should be able to tolerate the contaminants and to be adapted to the soils and climatic conditions of the area. In addition, in order to ensure the economical viability of the remediation processes, the use of energetic plant species for soil remediation is currently being deeply studied and under development.

The cultivation of lignocellulosic biomass with energetic application (bioenergy) in contaminated soils has numerous advantages, such as (Bernal et al., 2019): reduction in the competition with food production for bioenergy;low consumption of natural resources (soil and water);compliance with conservation objectives and environmental protection;increased food safety;replenishment of soil C reserves;improvement of soil health; low or zero cost of funding; and the use of native species as bioenergy crops, which would limit the introduction of non-native species into the soil ecosystem to be remediated.

2. SOILS AND THE ENVIRONMENT

Soil is the upper layer of the earth's crust, represents the interface between land, air and water and is home to most of the living organisms on the planet. Given the extremely long time it takes to form the soil (of the order of thousands of years), it can be considered to be a non-renewable resource (Jones et al., 2012). Many substances are stored, filtered and processed in the soil, including water, nutrients and carbon. Because of their biological, socio-economic and environmental importance, all these functions must be protected.

The soil originates from a parent material, generally bedrock, which is the source of the mineral content, as a consequence of the incessant action of meteoric degradation through different physico-chemical processes: crushing of the rock by the atmospheric agents or the roots of the plants; exposure to water, air and erosion; and interaction with microorganisms.

Soils are mainly composed of:- An inorganic fraction, normally the major component, consisting of solid debris (gravels, sands, etc.) containing minerals derived from the degradation of the bedrock or transported from different places, from chemical compounds in solution such as salts, oxides, hydroxides, etc., and colloidal clays;

- An organic, quantitatively minor fraction, which derives from the activity and decomposition of organisms that trigger mineralization processes, with the formation of organic compounds, and the humification that transforms plant and animal remains in humus, a colloidal mixture of predominantly organic substances (lignin, proteins, glucids, lipids, organic acids, etc.) in which no biological structure is longer recognizable;

-Water, which occupies the soil's interstices and comes mainly from atmospheric precipitation;

-Gas (O_2 and CO_2) in varying amounts, which occupies pores not filled by water; water and air confined to the empty spaces of the soil play a vital role for plants, also allowing the roots to breathe.

2.1. Soil quality

Defining and assessing soil quality is not easy and the approach has changed considerably over time. Soil is a living ecosystem and, therefore, the assessment of soil quality cannot be limited, as in the case of air or water, to its concentrationof pollutants.Soil quality can be defined more broadly as "the capacitity of a soil to function within ecosystem and land use boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health" (Bünemann et al., 2018).

Soil quality is related to the use of the soil. Therfore,and to carry out a precise assessment of the quality of a soil, the selection of soil quality indicators chosen based on their relationwith the functions of the soil or ecosystem services is required (Bünemann et al., 2018).These indicators can be divided into physical, chemical and biological; the most commonly used ones are (Bünemann et al., 2018):

- ➢ **Physical parameters**
    - o water storage capacity (water-holding capacity, watercontent, sorptivity, water-filled pore space,water retention, field capacity, permanent wilting point, plant-available water content, hydraulic conductivity of the saturated soil);

  - bulk and particle density;
  - texture (particle-sizedistribution,soiltexture(% of sand,siltand clay)).

- **Chemical parameters**
  - Total organic matter and total organic carbon, soil color and odor (if related to organic matter), organic matter humification (humic and fulvic substances and humification indices);
  - pH;
  - Soil salinity (electrical conductivity and soluble salts);
  - Macronutrients (P, K, Ca, Mg, K often aspart of nutrient availability or extractable);
  - Total N and inorganic forms including NH_4^+ and NO_3^-;
  - Cation exchange capacity and exchangeable cations.
- **Biological parameters**
  - Soil respiration, microbial activity;
  - Microbial biomass (microbial biomass, microbial C, microbial N, microbial P, bacterial biomass, fungal biomass);
  - C, N and P mineralization processes;
  - Soil macrofauna (including earthworms).

Extrinsic factors, such as those related to the climate, management or site data, are rarely mentioned, as well as those related to the yield, plant nutrient status and other measures of ecosystem services.Thisimplies that soil quality is usually not related to ecosystem services or soil threats. However, in some studies indicators such as earthworms, which serve as indicators for both the water and nutrient cycles, are taken into consideration (Lima et al., 2013).

Recent developments in soil biology highlight the fundamental role of soil-living organisms for its function.Such studies have focusedon the use of new or more efficient biological and biochemical indicators to improve the quality assessment of soil. Biological indicators are in fact necessary to link abiotic soil properties to changes in soil functions, with regard to biochemical and biophysical transformations (Lehman et al., 2015).Soil biota is considered the most sensitive indicators of soil quality, due to their very high reactivity to changes in environmental conditions (Bünemann et al., 2018). Molecular studies basedon DNA and RNA have a great potential to perform fast and economic measurements on processes between soil biota and the soil itself (Bouchez et al., 2016). All these indicators together allow a better and more detailed assessment of soil quality. However, it is necessary to keep in mind the practical use of these indicators by the different stakeholders.The practical limitations together with the absence of standardized operating procedures and accepted limit values, as well as the lack of functional connections with soil processes and management implications, make it difficult to apply these new indicators in routine assessments of soil quality (Callahan et al., 2016).

Concerning the chemical parameters, the total sum of the exchangeable cations (mainly Ca^{2+}, Mg^{2+}, K^+, Na^+, H^+, Al^{3+}) present on the surface of the soil colloids is called “cation exchange capacity” (ANPA, 2001).The CEC characterizes a soil not only from an agronomical aspect, i.e. for its fertility, but also from an environmental point of view, indicating its buffering capacity with respect to the presence of contaminats like, for example, heavy metals. The CEC is high in soils rich in clay and organic matter and can be considered as a substantially stable property. Also, soil pH influences the biological activity of certain functional groups of microorganisms that intervene directly in the biochemical cycles of different elements, such as nitrogen and sulphur. In addition, the pH has a more or less relevant influence on soil structure, as it affects the factors that determine the flocculation status of colloids (ANPA, 2001).

Regarding the physical properties, the useful depth is an indicator of the thickness of soil that can actually be explored by the root system and can be used by the roots of plants to draw water and nutrients from them (ANPA, 2001). In general, soil depth is assessed by identifying the contact between soil and unpedogenized geological substrat, whereas the “functional depth”or“useful depth”assessesin addition if some horizons are so dense and hardened to prevent theroot, or if there is free water, such as to induce asphyxia in most plants.

The gravitational water expresses the capacity of the soils to remove the excess water by percolation (leaching) or surface flow (ANPA, 2001).The permeability refers to capacity of the soil to allowthe air and water to move down the soil profile. It depends on numerous physical characteristics of the soil, such as the pores size, continuity and distribution, and properties, which are in turn related to the texture, the particle size and structure. Permeability is a soil property of priority interest and value for environmental purposes, since it is closely related to the hydrological behavior of soils and, therefore, to the risks of vulnerability of underground and superficial water.

The protective function of the soil expresses the ability of the soil to act as a barrier or a filter to the potential pollutants and to protect groundwater, surface water and food chain from contamination (ANPA, 2001). Soils, in fact, regulate the hydrological flows, controlling the transport of solutes in depth and the movement of the water on the surface, and promote the inactivation of the toxic substances through processes of adsorption, precipitation and biochemical and microbiological decomposition. The protective function of soils is relevant in the analysis of many environmental risks, such as contamination and eutrophication of water resources, erosion, compaction, flooding and acidification.

3. TYPES OF SOIL CONTAMINATION

Both polluted and unpolluted soils contain a variety of compounds that are naturally present, as metals, inorganic ions, salts (e.g. phosphates, carbonates, sulphates, nitrates) and countless organic compounds (e.g. lipids, proteins, DNA, fatty acids, hydrocarbons, PAHs, alcohols, etc.). However, pollution takes place when the quantities of soil contaminants exceed the natural (background) levels. Soil contamination can be defined as “the introduction of contaminants that can damage or destroy different soil functions and cause forms of indirect contamination” (Ashraf et al., 2014). There are, therefore, two main causes of soil pollution: natural causes and anthropogenic (artificial) causes. The natural processes leading to soil pollution are: the natural accumulation of compounds in the soil due to imbalances between atmospheric deposition and precipitation; natural production in the soil under certain environmental conditions (e.g. volcano eruptions); and losses from sewer lines to the subsoil. Human (anthropogenic) soil pollution originates in different types of processes, some voluntary (industrial) and others accidental.

The main sources of anthropological pollution can be diffuse, i.e. contamination of large areas due mainly to atmospheric deposition, flow and sedimentation; or localized contamination due to agriculture and horticulture, urban soils and industrial and/or mining contamination (Alloway, 2013). Soil contamination from diffuse sources is mainly associated with industrial emissions, vehicle traffic, energy production and waste treatment plants and the dispersion of pesticides, fertilizers, livestock slurry and sewage sludge in agriculture. In particular, the use (or misuse) of pesticides in agriculture has led to consider them as one of the main sources of diffuse soil pollution (Jones et al., 2012). The danger associated with the application of pesticides is given by their solubilization and consequent absorption, degradation and transport in the soil, which is the first receptor and the first and most important barrier to the penetration of pollutants to the aquifers. Another risk of pollution deriving from agricultural practices is dueto the use of farmeffluents (such as animal manures) in the soil, which can cause the overload of nutrients (NO_3^-) on the aquifers and accumulation in the soil of certain heavy metals present in animal manures (mainly Cu and Zn). Therefore, the composition of the surface layer of the soil is influenced by both local contamination and the transport of contaminants over long distances. Soil pollution from point sources, and therefore the presence of contaminated sites, represents a degradation of soil quality that prevents the proper development of the soil functions.

Contaminants present in the soil can be dangerous even atvery low concentrations for both human health and the environment. In fact the toxicity of a soil is not necessarily determined by the total concentration of the contaminants as phytotoxicity may be absent at high total concentrations, while biological processes may be compromised by levels of trace elements lower than the maximum permitted levels (Clemente et al., 2015). In addition, the simultaneous presence of multiple contaminants in the soil may cause interaction effects (sinergism or anthagonism) and amplify their negative influence.

3.1. Trace elements

Inorganic contaminants include metallic elements, comprising the usually called heavy metals, like cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), and (Zn), and metalloids, like arsenic (As), antimony (Sb) or selenium (Se), with some characteristics similar to metals and others to non-metals. Metal(loid)s are elements that can be found naturally in soil. Some of these (e.g. Fe, Mn, Zn, Cu, Ni and Mo) are essential plant nutrients (Dal Corso et al., 2013), while others, such as Cd, Pb and Hg, have no biological function (Tangahu et al., 2011). The whole group can be termed potentially toxic trace elements (TEs), because their toxicity is not absolute, on the contrary, highly dependent on different factors (e.g., total concentration, bioavailability, environmental conditions, target organism). These elements originate both from natural sources, such as the pedogenetic substrate, and from anthropogenic sources such as industrial, civil and agricultural activities. The TEs originating from the pedogenetic substrate are considered as geochemical pollutants, which can produce biological damage and are normally limited to restricted areas. The TEs resulting from industrial activities are mostly Zn, Cu, Pb, Cr, Ni, Cd and Hg, while those coming from civil activities like fuels used for heating and exhaust gas from vehicle traffic are mainly Pb, Cd and Zn (ANPA, 2001). Some agricultural activities can be a source of pollution with TEs to the soil. More than 10 % of the fungicides and insecticides used in the past provided As, Cu, Hg, Mn, Pb and Zn to the soils. Pig slurry may contain significant amounts of Zn and Cu that originate from the animal’s diet supplements (Moral et al., 2008; Clemente et al., 2020). Finally, some fertilizers can contain As, Co, Cr, Cu, Mn, Mo, Ni, Pb and Zn, which may derive both from the raw materials and from industrial processes. Among these, perfosphates are particularly suspects for their possible contribution of Cd and Pb (ANPA, 2001). One of the main sources of metal contamination is undoubtedly mining, in particular, the residual fraction resulting from the extraction process. These residues are characterised by a high presence of particles belonging to the silty and sandy fraction, low content of biological nutrients (such as N, P, K) and organic matter (Mendez and Maier, 2008). Trace elements, unlike other contaminants, cannot be degraded, can have harmful effects on biological systems and often accumulate there. They can cause negative effects on biological systems even if present in very low concentrations. In plants, they can alter development, the ability to create plant cover and have a negative impact on soil microflora (Tangahu et al., 2011). Typically, the following concentrations of metals and metalloids can be found in plants (Van der Ent et al., 2013): Ni (1.5), Zn (50), Cd (0.05), Pb (1), Cu (10), Co (0.2), Cr (1.5), Mn (200), Tl (0.02), As (0.1) and Se (0.02) ($\mu g\ g^{-1}$).

Some TEs (mainly metals) are enzymatic cofactors and are involved in important metabolic processes. For example, Mn and Cu are essential for photosynthetic activity, others such as Zn are involved in the transcription of DNA, Ni in the hydrolysis of urea into carbon dioxide and ammonia, and Co and Zn in the formation of legume nodules and are therefore important for nitrogen fixing (Vamerali et al., 2009). The interactions that can occur between metals and macronutrients, as regards absorption and transport, depend on the concentrations in which they are found in the soil. Phytotoxicity is often associated with non-essential TEs such as As, Cd, Cr and Pb. These elements, with the exception of Cr, are not essential for humans and can enter the food chain through different foods. In general, As, Co, Cu, Cr, Pb, Hg are not easily transferred into aerial plant biomass (Amer et al., 2013; Kumar et al. 1995; Tangahu et al., 2011; Vamerali et al., 2009), but rather accumulated mainly in root cells, while Cd, Ni, and Zn show a fair mobility (Trakal et al., 2015). A very toxic TE for plants and human health is mercury; in fact, in the metallic form of Hg damages photosynthetic activity and oxidative metabolism, because it interferes with the electron transport in chloroplasts and mitochondria. Furthermore, Hg inhibits the activity of aquaporins and reduces water absorption by plants (Tangahu et al., 2011). Another highly toxic TE is As, some of its compounds have been used in the past as herbicides; it is generally phytotoxic at concentrations $<20\ mg\ kg^{-1}$ in the whole plant. However, there are species such as *Pteris vittata* L. capable of accumulating concentrations> 1000 mg kg^{-1} in the aerial part only (Tangahu et al., 2011; Vamerali et al., 2009).

The toxicity of anelementis related to its mobility (i.e., itsability to migrate to deep soil layers with the risk of contamination of groundwater), and its bioavailability (i.e., the ability to be absorbed by the vegetation and/or soil living organisms), with the direct risk of entering the food chain (Dominguez et al., 2017).Within the soil system, TEs can be present in different forms:as ions dissolved in the soil solution; forming insoluble salts;adsorbed on colloids (clays and/or organic matter); as elements of plant tissues;and as components of the crystalline lattice of minerals. The most dangerous forms are therefore the soluble and adsorbed ones, as these can easily move through the soil profile and are easily accessible to plant roots and soil living organisms (Clemens 2006; Morgan, 2013).

The bioavailability of TEs in contaminated soils depends on different physical, chemical and biological factors (Committee on Bioavailability of Contaminants in Soils and Sediments, 2002).

- ➢ **Physical:** the texture and structure of the soil influence the distribution of metals in the various soil horizons. The presence in the soil of neo-forming compounds such as clay minerals, iron and alluminium oxides/hydroxides, makes the soil capable of adsorbing and retaining heavy metals and metaloids. The affinity between clay minerals and TEs depends on the colloidal nature, and the number of surface charges able to actively retain ions. A very coarse structure, on the other hand, makes most of the metal inaccessible to the roots of plants (De Vivo et al., 2004).

- ➢ **Chemical:** conditions such as soil acidity and redox status can influence the bioavailability of TEs. For instance, some metals are mobilized in acidic conditions, while other TEs (such as As) are mobilized under alkaline conditions. High levels of redox potential (Eh) are typical of dry and well-aeratedsoils; conversely, soils rich in organic matter and under submerged conditionstend to have reduced conditions with low Eh. This last condition can promote solubilization, and a consequent greater bioavailability of metals (Petruzzelli and Pedron, 2007). The organic matter (OM) level of the soil and its soluble fraction and humification degree can affect the solubility, availability and toxicity of TEs in the soil (Bernal et al., 2009).

- ➢ **Biological aspects**: soil biota, in particular bacteria, fungi and higher plants, can strongly modify the chemical and physical conditions of the soil and the processes that determine the bioavailability of TEs. For example, in mining soils, chemolithotrophic bacteria can acidify the soil and therefore increasethe mobility of the TEs, or even provoke their precipitationin the form of sulfides

(Ernst, 1996).The symbiotic association between fungi and roots (mycorrhiza) in soils contaminated with TEs causes the increase of mycorrhizosphere volume, compared to the rhizosphere alone, providing greater access to soil resources including macro-, micro- and even non-essential elements. Because of these changes, it can be expected that the entire microflora around the root (rhizosphere) influences TEs speciation and therefore their bioavailability in the soil (Audet and Charest, 2009; Martínez-Alcalá et al., 2009).

Agricutural practices, such as the addition of organic (compost, biosolids as sewage sludge, animal manure manure and slurries, peat, municipal solid waste, etc.) and inorganic (liming, by-products of industrial activities) amendments can be avery effective way to reducethe bioavailability and toxicity of TEs in contamined soils (Bernal et al., 2009; Puschenreiter et al., 2005). In fact, the organic matter from organic amendments can bind TEs such as Cu, Fe, Mn and Zn, transforming them into insoluble forms. The addition of organic matter (OM) to contaminated soils may influence TEs fractionation through the following mechanisms:

- Mobilization of the TEs by complexation with soluble OM (mainly for Cd, Zn and Ni);
- Decomposition of the OM to which they are linked, releasing them into the soil solution;
- Formation of metal complexes;
- Retention in the exchangeable sites;
- Adsorption to colloidal particles of OM.

3.2. Soil nutrients

The increase in nutritional requirements to feed the growing population has led to the need for addition of nitrogen (N), phosphorous (P) and potassium (K) to soils through both synthetic and organic fertilizers. However, over application or inadequate use of fertilizer products can lead to a reduction in nutrient use efficiency and, in turn, a reduction in crop yields (Hossain et al., 2005; Zhu et al., 2005; Hao et al., 2003). The excessive supply of N and P is the cause of nitrate pollution in water. In fact, these nutrients are able to penetrate into groundwater or be transported by surface run-off to water bodies, causing their eutrophication and significant environmental (Good and Beatty, 2011; Vitousek et al., 2009; Withers et al., 2014) and human health concern (EC, 1991; Frumin and Gildeeva, 2014; Pretty et al., 2003; Yaron et al., 2012). Furthermore, the presence of some TEs has been detected in some P and N fertilizers, including As, Cd, Cr, Hg, Pb and Zn (Steffan et al., 2018). Phosphate fertilizers are, among all mineral fertilizers, the major source of TEs, as these are normally present in the phospahate rock that is used as the source of P for P-fertilizers (McLaughlin et al., 1996).

3.3. Pesticides and POPs

The term “pesticides” is commonly used as a synonym for plant protection products. The term “pesticides” is, however, a broader term that also includes products such as biocides, which are not intended for use on plants, but serve to eradicate harmful organisms and carriers of diseases (https://www.efsa.europa.eu/it/topics/topic/pesticides). Plant protection products are pesticides used to keep crops healthy and prevent them from being affected by diseases and pests. They include herbicides, fungicides, insecticides, acaricides, plant growth regulators and repellents. These products contain at least one active substance, which can be chemical substances or micro-organisms, including viruses, which allow the product to perform its action (USEPA, 2014).

Some pesticides are also persistent organic pollutants (**POPs**), i.e. chemical substances that persist in the environment, bioaccumulate through the food network and pose the risk of causing adverse effects on human health and the environment. POPs include chlorinated and brominated aromatic compounds (such as polychlorinated biphenyls, PCBs) and organochlorinated pesticides (such as DDT), as well as industrial chemical substances and by-products of industrial processes (such as dioxins and furans) (US EPA, 2014). POPs form stable bonds with soil organic matter, where they remain in a non-extractable form. However, changes in environmental conditions can alter the amount of POPs in the soil. For example, the increase in temperatures produces an increase in the volatilization of POPs from soils (Komprda et al., 2013), while low temperatures favor their deposition (Guzzella et al., 2011).

Polycyclic aromatic hydrocarbons (**PAHs**) are a group of persistent, semi-volatile organic pollutants (e.g., fluoranthene, naphthalene, pyrene, phenantrene and benzopyrene; Lerda, 2011). Most PAHs, due to their persistence and hydrophobicity, tend to be retained in the soils for a long period of time and, for this reason, they can be also considered POPs. They are widespread in air, water, soils and sediments (Lin et al., 2013). PAHs are relatively soluble in water and organic solvents, they accumulate in the lipid tissues of plants and animals, but they do not tend to accumulate in the tissues of plants with a high water content. In general, there is a limited transfer from the soil to plants (vegetables) roots (Abdel-Shafy and Mansour, 2016).

3.4. Radionuclides

The most common radionuclides found in soils are ^{40}K, ^{238}U, ^{232}Th, ^{90}Sr and ^{137}Cs (Wallova et al., 2012). The sources can be both natural or anthropogenic, but the majority derives from nuclear pollution due to nuclear weapons tests, from the management of nuclear waste and disposal, from the extraction of radioactive minerals (Ćujić et al., 2015) and from nuclear accidents, as for instance Tre Mile Island, United States of America (1979); Chernobyl, Ukrainian SSR (1986); Goiânia, Brazil (1987); Tokaimura (1999) and Fukushima (2011) in Japan.

3.5. Emerging pollutants

Emerging pollutants (**EPs**) refer to a large number of synthetic or naturally occurring chemicals that have recently appeared in the environment and are not commonly monitored (Geissen et al., 2015). Because the sources of EPs are different, their nature, physic and even chemical properties are different, including their volatility, polarity, adsorption, persistence and interaction with the environment. The main EPs that affect soils are:

- Pharmaceutical and personal care products (**PPCP**): pharmaceutical and chemical products including cosmetics, perfumes, food supplements and additives used in many household cleaning items. They are biologically active compounds (Boxall et al., 2012), designed to interact with hormonal processes or living tissues; therefore, it is necessary to know the fate, the effects and the potential risk when they are released in the environment. Studies have proven the chronic toxicity to soil and aquatic organisms (Chalew and Halden, 2009), and the presence of PPCP has been linked to the development of antibiotic-resistant bacteria, feminization of male fish and genotoxicity of aquatic organisms (Costanzo, et al., 2005); Medicinal and veterinary products are present in the environment deriving from human and livestock treatment to promote growth and disease reduction or prevention. These substances are absorbed and undergo metabolic reactions (e.g., hydroxylation, splitting or glucuronation) to produce metabolites, which may be even more harmful than the original compounds (Díaz-Cruz and Barceló, 2005). Furthermore, large quantities of drugs are not assimilated or metabolized and can return to the environment via faeces and urine in urban wastewater and animal manure. Then, the application of sewage sludge or animal manure to agricultural soils as fertilizer products, or when treated wastewater is used for agricultural irrigation, crops are exposed to antibiotics that can persist in soils for several to several hundred days. Studies have shown that some antibiotics, particularly tetracycline, amoxicillin and fluoroquinolones, can be absorbed by cultivated plants (Azanu et al., 2016);

- Plasticisers are additives used to increase flexibility or plasticity, such as bisphenol A (**BPA**) or phthalate esters (**PAE**) that are particularly recognised as endocrine disrupters (Ghisari and Bonefeld-Jorgensen, 2009). These polymers can befound in products such as lubricating oils, car parts, paints, glues, insect repellents, photographic films, perfumes and food packaging. Both PAE and BPA have been detected in food and humans and are listed as toxic agents in different international regulations (Rodriguez-Eugenio et al., 2018; Yen, Lin-Tan and Lin, 2011); BPAs in particular have already been banned or strictly regulated (Rodriguez-Eugenio et al., 2018).

- Two other major groups of emerging contaminants are manmade nanoparticles (**MNPs**) and treatment by-products. They are present in over a thousand products, also as additives for paints, cosmetics, fabrics, papers, plastics and food products (Rodriguez-Eugenio et al., 2018), in fabrics to produce self-cleaning, water-repellent and antimicrobial and abrasion-resistant clothing. Artificial nanoparticles have toxic effects on organisms through mechanisms of: generation of reactive species of oxygen (which cause oxidative stress); alteration of the permeation properties of membrane cells (thus interfering with physiological activities); alteration of the process of electron transfer and; finally, modification of the conformation of proteins (interfering with the transfer of bio-signals and gene formation (Pan and Xing, 2012).

- **Plastics and microplastics**. The main source of plastic contamination in soil is the degradation of plastics used in agriculture (from greenhouses, tunnels, mulch, irrigation systems, silage and packaging; Rillig et al., 2017). Although plastic debris can enter the soil through the application of low quality municipal solid waste (Weithmann et al., 2018), sewage sludge (Corradini et al., 2019) and irrigation (Mohapatra et al. 2016). Among the different uses of plastic, mulching is the more likely to lead to plastic contamination in soil (Hayes et al., 2017). Over the last few years, new biodegradable mulching films, either biobased or synthetic or a blend of both, have been developed (Sintim and Flury 2017). The actual information available regarding degradation rate of biodegradable plastic in the soil is controversial as biodegradation standard tests are often carried out in optimum (unreal) environments, with constant humidity, temperature and oxygenation (Napper and Thompson, 2019).

4. LEGISLATION ON CONTAMINATED SOILS

In Spain, the policies related to the recovery of contaminated sites started in the 1990s. In 1995, the Ministry of the Environment approved the National Plan for the Recovery of Contaminated Soils (1995-2005), which set out a number of objectives for the management mechanism and co-financing with the autonomous regions, which constitutes the general framework for the recovery of contaminated sites in Spain. In 1998, the law on waste (Law 10/1998, of 21 April on waste) was approved and repealed by the law of 28 July 2011. It applied to all types of waste, with the exception of air pollutant emissions, radioactive waste and discharges into waterways. One of the fundamental principles of the law was the introduction of a policy of “prevention”of waste, i.e. the regulation of the production phase and the introduction on the market of products that, once used, generate waste materials. The autonomous communities were also asked to maintain an inventory of "contaminated land" and to implement all the procedures necessary for their recovery.

In 2005, the implementing regulation Royal Decree 9/2005 of 14 January 2005, was adopted in Spain, describing the relationships between potentially soil polluting activities and the criteria and standards for the declaration of contaminated soils. The decree uses the generic category of reference levels (NGR), including the concentration of a pollutant in the soil that does not imply a higher risk than the maximum acceptable for human health or the ecosystem. Starting with NGRs, autonomous communities may decide to declare a contaminated site if the NGR is exceeded, or to require a specific risk analysis to be carried out for the site. However, they may consider the potential risk low enough to require no further action. The decree considers three types of land use: industrial, residential and natural.

In 2009, the National Integrated Waste Plan for the period 2009-2015 (Plan Nacional Integrado de Residuos para el periodo 2008-2015) was approved in Spain, part of which is dedicated to contaminated soils. Subsequently, Law 22/2011 of July 28, 2011 transposed into Spanish law the EU Directive 2008/98/EC on waste, which established a comprehensive legal framework, at European level, on waste management. Law 22/2011 oriented waste policy towards the principles of maximum use of resources, on the one hand, and minimizing environmental impact by introducing measures for the prevention, reuse and recycling of waste on the other hand, and promoted scientific and technological innovation to reduce emissions of harmful gases into the atmosphere.

At the moment, a new legislation concerning wastes and contaminated soils is under discussion in Spain, in order to adapt the actual legislation to the requirements of the European Circular Economy Package. The new law includes the EU Directive 2018/851/EC, which modified the EU Directive 2008/98/EC on waste, and the EU Directive 2019/904 related to the reduction of the impact of several plastic products in the environment. The regulation concerning contaminated soils maintains the potentially contaminant activities described in the previous legislation, including the procedure to declare a soil contaminated, and the inventories of contaminated soils at national and regional levels. Also, the responsabilities for decontamination and remediation of a contaminated soil are updated and a new National Inventory of voluntary decontamination of contaminated soils is included.

5. PHYTOREMEDIATION

Phytoremediation techniques (Figure 2.1) were born at the beginning of the 90's and today, still in an experimental phase, they tend to establish theirselves as a consistent an effective restoration approach. They consist of a series of technologies used for environmental restoration and are based on the ability of some plant species to assimilate, accumulate, immobilize and degrade contaminants, such as trace elements, organic compounds and radioactive elements present in soil and groundwater (EPA, 2012). This method of rehabilitation exploits different phenomena that occur in the soil-plant system, including the complex interaction between the roots of the plants and soil microorganisms (Kidd et al., 2009).

The contaminants that can be addressed by these technologies are numerous: trace elements (including metals like Cd, Cr, Pb, Co, Ni, Se, Zn, Ag and Hg and metalloids like As and Sb), radionuclides (Cs, U, Sr), chlorinated solvents, hydrocarbons, chlorinated pesticides, organophosphate insecticides, herbicides, explosives, emulsifiers, polychlorinated biphenyls and organic compounds. Phytoremediation is based on natural processes carried out by plants and their associated microorganisms, which include: direct absorption of TEs and some organic compounds; accumulation or transformation of the same chemicals by lignification, metabolisation or volatilization; use of enzymes released by plants to catalyze the degradation of organic pollutants; release of exudates in the rhizosphere, leading to anstimulation of the microbial activity and the immobilization or degradation of the pollutants.

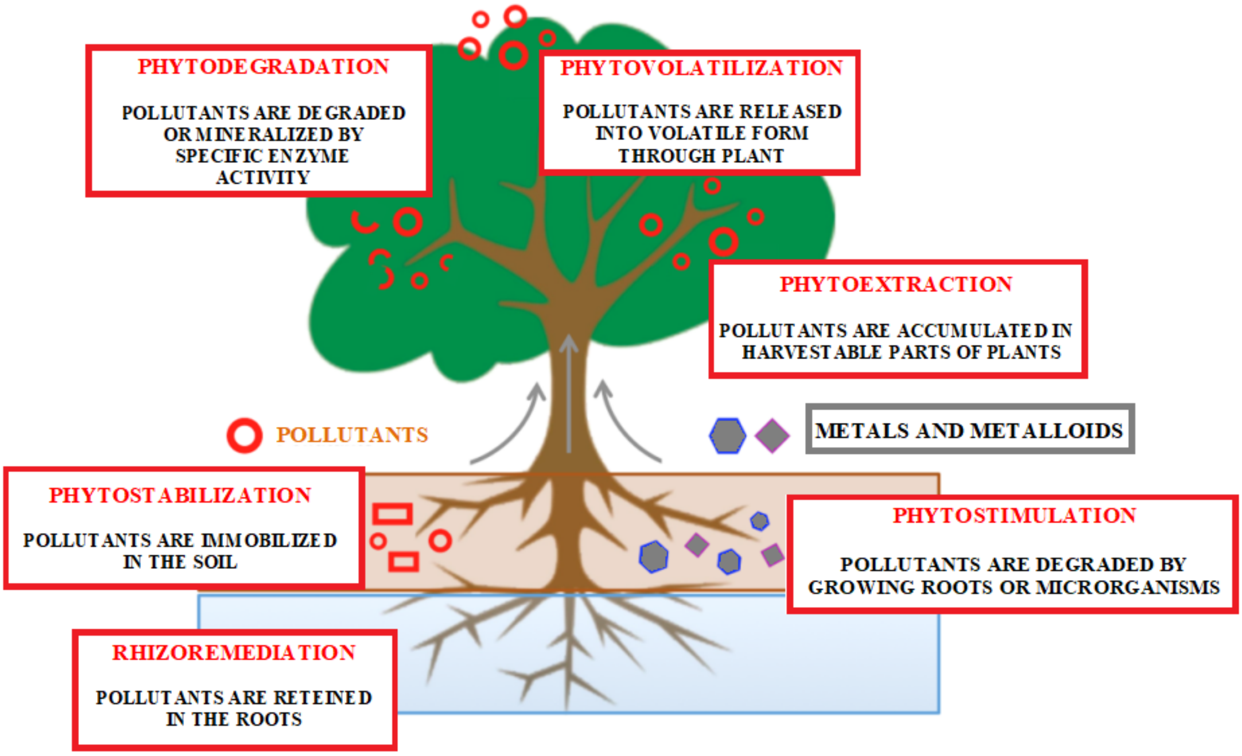

Figure 2.1. Phytoremediation processes.

Phytoremediation techniques can be divided into two categories: “in-situ” and “ex-situ”. The in-situ interventions allow the decontamination of affected areas on site, i.e., without removing the soil or water to be reclaimed. This method is therefore preferred to the ex-situ ones both from an environmental, agronomic and economic point of view (Quintella et al. 2019). Ex-situ interventions are used only when the in-situ methods cannot guarantee sufficient levels of remediation, or when, due to the particular characteristics of the contaminated matrix or the high concentration of pollutants, the excavation of the soil and its subsequent treatment becomes necessary even at a considerable distance from the place of intervention. This certainly requires costs and a much higher environmental impact linked to the numerous operations of removal and transport (Zerbi and Marchiol, 2004).

Phytoremediation, depending on the reclamation mechanism involved, can be divided into different types, namely phytoextraction, phytostabilization, rhizodegradation, phytodegradation or phytotransformation, phytovolatilization and rhizofiltration (Salt et al, 1998; Cunningham et al., 1995; Miceli, 2001; Pulford and Watson, 2003; Wong, 2003; Zerbi and Marchiol, 2004; Mertens et al., 2004; Rizzi et al., 2004; Kramer, 2005). The type of mechanism of action to be used will depend on the type and degree of contamination and the objectives of restoration (containment, stabilization, isolation, assimilation, reduction, detoxification or degradation of pollutants). In accordance with these objectives, the selection of the most appropriate phytoremediation strategy cannot ignore the adequate knowledge of the site characteristics from the soil, climate and hydrological point of view (Barbafieri, 2005).

5.1. Phytoextraction

The phytoextraction of heavy metals and metalloids consists in the absorption of contaminants through the root system and the subsequent translocation and accumulation in the above ground parts of the plant (shoots, leaves, stems), which are then harvested and the contaminants removed from the soil. Different processes are involved in this technology:

- compartmentalization, i.e., segregation of the contaminants in the vacuoles;
- chelation, which allows pollutants to be irreversibly bound to organic molecules such as phytochemicals or citric acid, or to proteins present on the cells of the root surface;
- biotransformation, which transforms the contaminats into biologically less active and therefore less toxic forms.

The useful plant species for this technology should be tolerant to the contaminant by accumulation (accumulators/hyperaccumulators; see section 6.3). Phytoextraction can be successfully applied in soils with shallow contamination so the roots have direct access to the contaminants, and soil concentrations are just above thresholds or guide levels (Dickinson et al., 2009).

Therefore, two different phytoextraction approaches exist (Sas-Nowosielska et al., 2004):

1) **Continuous phytoextraction**: that uses hyperaccumulator plant species which are able to absorb and accumulate high concentrations of pollutant during the entire life cycle, although they usually produce little biomass.

2) **Induced or assisted phytoextraction**: that uses species (mainly crops) with high biomass productivity, capable of absorbing high amounts of the contaminant for a limited time, after it has been mobilised in the soil by a chelator. The bioavailable fraction of the contaminants in the soil is that present in chemical forms that can be absorbed by the plant, which is often only a small fraction of the total present in the soil. However, the addition to the soil of chelating agents (EDTA, oxalic acid, citric acid), which form water-soluble compounds with the TEs, increases their solubility and availability and therefore, can potentially increase their absorbtion by the plants and their accumulation in the aerial part at a high level (Suthan Suthersan, 2002).

5.2. Phytoimmobilization and phytostabilization

Phytoimmobilization or phytostabilization refers to the process by which plants stabilize the contaminants through their immobilization in the rhizosphere (the part of the soil in contact with the roots) thanks to the production of roots exudates, or in the roots, thus reducing their mobility and their bioavailability. These technologies prevent the migration of the contaminants into groundwater or, ultimately, entering into the food chain, without the contaminants themselves being removed from the soil (Suthan Suthersan, 2002). Phytoimmobilization leads to the transformation of soil TEs into less toxic forms, but not to their removal from the soil. Phytostabilization refers to the physical retention of the contaminants through the action of the root system, to prevent the contaminant from migrating to the underground layers or dispersing into the environment (Chaney et al, 1997). Tolerant plant species by exclusion are used for these technologies.

5.3. Rhizodegradation

Rhizodegradation is the degradation of organic contaminants that occurs in the rhizosphere. The technique is based on the symbiotic relationship established in the soil between microorganisms (bacteria, fungi and yeasts) and the plant root system, which is able to exude certain compounds (sugars, amino acids, fatty acids, phenols, etc.). Enzymesand lysates (substances released by cell lysis) allow the establishment of favourable conditions (e.g., acid pH conditions) for the development and growth of communities of microorganisms capable of metabolizing a wide variety of organic contaminants such as hydrocarbons, PAHs, BTEX, pesticides, chlorinated solvents, PCBs and surfactants (EPA, 2000). Since contact between the root system and the contaminated matrix is essential for the success of this technology, root extension, in terms of density and depth, plays a key role in the treatment of sites contaminated with organic compounds (EPA, 2000).

5.4. Phytodegradation

Phytodegradation or phytotransformation consists in the absorption of contaminants, which are accumulated in the plant tissues, and then degraded through natural metabolic processes. Complex organic molecules are reduced to simple organic molecules and incorporated into plant tissues through internal processes of lignification, or they can be mineralized to carbon dioxide and water, thus promoting plant growth (EPA, 2000).

5.5. Phytovolatilization

Phytovolatilization consists in the absorption of the contaminant from the soil and, once in the plant, is transported to the leaves and from there through the stomata from which is released into the atmosphere by transpiration. This technique is useful for volatile inorganic and organic contaminants such as benzene, chlorinated solvents, As, Hg, Se (EPA, 2000).

5.6. Rhizofiltration

Rhizofiltration is the uptake by the root system of the pollutants present in dissolved form in groundwater or contaminated water. It occurs mainly in the root zone, through processes of adsorption, concentration or precipitation of the contaminants. Consequently, this process is not used for soil remediation but rather for the treatment of water and wastewaters, but could be used in the presence of marshy or stagnant soils. This technique essentially consists of using plants as a hydraulic barrier against contaminants dissolved in the water, favouring their precipitation. Usually, this technique is used for ex-situ treatments, where the water is transferred to artificial hydroponic systems (wetlands), in which the plants grow with virtually immersed roots and once the roots are saturated with contaminants they are collected, dried and sent to the final process. This mechanism has been shown to be useful to accumulate mainly heavy metals such as Pb, Cd, Zn, Cr, Cu and radionuclides such as Uranium 234 and Cesium 137 (EPA, 2000).

6. FACTORS INFLUENCING PHYTOREMEDIATION

Phytoremediation effectiveness depends on a large number of variables and it is a site-specific technique. Themost remarkable variables that condition these processes are:

- the type and concentration of the contaminants and depth of contamination;
- environmental characteristics (climatic and soil conditions) such as the hydric status, mineral composition, pH of the soil and all chemical and physical aspects affecting the availability of the contaminants;
- the requirement of soil organic/inorganic amendments;
- the plant species.

6.1. Contaminants and soil conditions

The type of contaminant and the interactions between the different contaminants determines the most recommended remediation technology. In fact, often the sites are contaminated by several elements or type of contaminants. For instance, most of the studies on phytoextraction have been performed with plants in matrices polluted by a single element, even if some species seem to tolerate several substances simultaneously. However, there is still not enough experimental evidence to prove the efficiency of remediation in pluri-contaminated soils. It is assumed that in multi-contaminated soils it would be necessary to carry out integrated treatments involving different species simultaneously or in rotation, depending on their specific mechanisms of action (ITRC 2009; Kidd et al. 2015).

The concentration of the contaminant also influences the outcome of phytoremediation, because exccesively high concentrations can be toxic for the plants, reducing the absorption potential by inhibiting the growth and survival of the plant and therefore the success of the intervention. This is because each plant species has a specific level of tolerance for a certain contaminant, above which the physiological mechanisms of the plants to cope with the contaminant are overcomed (Clemens et al., 2002).

Moreover, the depth of the contamination affects the success of the phytoremediation. Large areas with a surface contamination are easier to reclaim than small areas with a deep contamination, as the plant roots cannot penetrate to those layers. The use of trees for phytoremediation is a good option for deep contamination (EPA, 2000; Gómez, et al. 2019).Most studies to date have focused on the genus Populus and Salix, as they can virtually consider ideal trees for phytoremediation, due to their characteristics such as: pioneer, fast growth, high transpiration, big roots, natural tolerance to pollution and stress, economic and ecological values (Gómez et al., 2019).

As previously indicated, low soil pH values favour the solubility of most TEs, which can therefore be easily absorbed by the plants. Such solubility increases the risk of leaching down the soil profile and thus reaching the underground aquifers causing their pollution.The pH of the soil is decisive both for the physiological development of the plants and for life in the soil in general. A soil that tends to be acidic would therefore be favourable for phytoextraction interventions. In fact, soil acidification resulting from the use of ammonium sulphate, used as a fertilizer, is capable of favouring mobility and the assimilation of Cd and Zn (Chaney et al., 2000). Several studies indicated that, after the process of phytoextraction carried out in soils with acid reaction and thanks to the activity of plants and the favorable actions exerted by microorganisms in the rhizosphere, the soil can be returned to agricultural activity and to the normal development of the ecosystem, possibly bringing the pH back to a level close to neutrality (Chaney et al., 2000).However, liming can reduce the mobility and toxicity of TEs to plants, as lime can in fact increase the ability of the soil to bind metals, and their precipitation in insoluble forms, reducing their mobility and therefore the potential for phytoextraction, but improving the conditions for phytoimmobilization or phytostabilization.

Phosphorus, for example, is an essential macronutrient for plants, and P-fertilization is basic for increasing the biomass production and the development of the root system. However, the addition of phosphate fertilizers can also inhibit the absorption of some contaminants such as Pb and other heavy metals, due to their precipitation as metal phosphates (Chaney et al., 2000). One possible solution could be through foliar P-fertilizer applications, to guarantee the nutrient to the plant without interfering with the mobility of the metals.

Soils and sediments have a certain capacity to store and immobilise chemical substances, but this can vary when the factors that can influence their storage capacity and, therefore, modify their bioavailability with the consequent mobilisation in the environment, intervene ((Narasimha et al., 2020). The capacity of soils to adsorb and immobilize TEs is strongly influenced by properties such as pH (as already discussed) redox potential, salinity and organic content, which in turn directly affect the cation exchange capacity and soil structure (Zhang et al., 2014; Diacono and Montemurro, 2015; Wang et al., 2014). The above properties are strongly influenced by temperature and precipitation and thus the moisture content of the soil (Karmakar et al., 2016), which affect the kinetics of microbial processes that determine the organic content of soils (Karmakar et al., 2016; Stigliani et al., 1991). An increase in soil moisture leads to an increase in the organic matter content, a decrease in dissolved oxygen content and thus low redox potential values (Husson, 2013; Schulz et al., 2015), influencing the mobility of TEs (Narasimha et al., 2020).However, high temperature and low humidity conditions make the soils drier, more porous and aerated; microbial decomposition increases with a decrease in organic matter, and the soils are richer in oxygen and therefore have a high redox potential (Schulz et al., 2015; Husson, 2013). Furthermore, there is an increase in salinity when the evapotranspiration rate is higher than the precipitation rate.

Moreover, decreasing the organic matter content led to reduced cation exchange capacity of the soil, limiting the capacity of the soil to adsorb TEs (Narasimha et al., 2020). High redox potential leads to a greater mobilization of metals in the form of soluble sulphates and finally the increase in salinity reduces the storage capacity of TEs (Acosta et al., 2011).This means that the increase in temperatures due to global warming could significantly reduce the capacity of soils to store TEs (Wilson and Bell, 1996; Acosta et al., 2011), and thus increase their bioavailability.

6.2. Organic and inorganic amendments

Soil amendments can affect the solubility, toxicity and distribution of TEs in the soil. The effects depend both on the characteristics, properties and chemical composition of the amendment, andon the characteristics of the soil and the specific contaminants present. Therefore, the selection of an appropriate amendment for a particular site is a crucial step in assisting the phytoremediation process (Bernal et al., 2009).

Contaminated soils often present low fertity and have a low content of organic matter. This leads to a scarce presence of microbial biomass and activity, poor nutrient availability and, moreover, there is often a limited or almost non-existent vegetation cover. It has been demonstrated that the addition of exogenous OM has beneficial effects on soil fertility, affecting positively the physical, chemical and biological soil properties (Bernal et al., 2009). The most important are:

- formation of stable aggregates, which improve water permeability and porosity;
- improvement of the soil capacity to retain water, increasing the amount of water available to the plants and favoring seed germination;
- increase of the cation exchange capacity (CEC), which favors the adsorption of some nutrients reducing their loss by leaching;
- gradual release of mineral elements, through a slow mineralization, which thus represents a source of N, K, P and S for plant growth;
- a source of organic C and energy for soil microorganisms involved in the transformations that take place in the soil itself.

Therefore, the correct choice of the amendments considering the soil characteristics is a fundamental step in the phytoremediation process. The beneficial effects of some organic soil amendments used in phytoremediation of TEs contaminated soils have been already reported (Bernal et al., 2009; Clemente et al., 2015; Pardo et al., 2017):

➢ Animal manures are used in phytostabilization, as they normally decrease Cd, Ni, Pb, Zn solubility and enhance fixation into non-extractable forms in the soils; they can increase soil pH and reduce Eh;

➢ Peat can be used in phytoextration, as it increases soluble and exchangeable forms of Cd, Ni and Zn in soil, due to its acidic pH and its content of humic substances, and enhances Zn accumulation in the plants;

- Composted municipal solid wastes can be used in soil restoration, as they can reduce Cd, Cu, Zn leaching;

- Sewage sludge is used in stabilization and immobilization, decreasing Cd, Pb and Zn solubility and leaching through their accumulation in the least-available soil fractions;

- Compost (well-matured from different sources – olive mill wastes, animal manures, etc.) is used in stabilization and immobilization, through reducion of water-soluble Pb and Cd and plant Pb uptake, but a temporary increase in exchangeable Zn can occur shortly after soil application;

- Olive-mill wastes can be useful for phytoextraction, as they tend to increase soil Mn solubility and the metals associated with Mn oxides leading to metal accumulation in plants.

Recent studies have shown the effectiveness of different combinations of organic and inorganic soil amendments (such as solid fraction of pig slurry with paper-mill sludge, lime or a commercial derivative of red mud) for the phytostabilization of acid mine soils contaminated with TEs (Clemente et al. 2019; Pardo et al ., 2014). The combinations of amendments tested significantly increased soil pH (up to 5 and 6) and concentrations of total organic Cand total N, allowing adequate growth and development of the plants studied, such as native species from southern Spain (*Silybum marianum* and *Piptatherum miliaceum*), while the same plants were not able to grow in untreated soils. The aim of combining organic and inorganic soil amendments is to improve simultaneously different soil properties, such as the increase of soil pH in acid soils or improving As adsorbtion capacity of the soil through the addition of Fe-oxides, and at the same time improve soil fertility through the OM added by the organic amendment. Such combinations led to a significant stabilization of the contaminants in the soil, reducing the concentrations of their soluble and highly mobile chemical forms in the soil (the most available for plants).

Yang et al. (2012) found that both organic acids with low relative molecular weight (like acetic acid and citric acid), ethylenediamine tetraacetic acid (EDTA), sepiolite and fosfogypsum have different effects on plant growth when added to TEs contaminated soils. Water-soluble chelates of certain TEs (Schmidt, 2003) can be particularly useful in their mobilization in high pH soils for their phytoextraction (Rodriguez Eugenio et al., 2018). Furthermore, chelates facilitate TEs translocation from the roots to the shoot (Shen et al., 2002; Santos et al., 2006), especially in the case of Pb-EDTA complexes (Sarret et al., 2001), Pb-EDDS (Tandy et al., 2006), Cu-NTA (Wenger et al., 2002), Cu-EDDS (Luo et al., 2005) and Zn-EDTA (Collins et al., 2002). Some studies have been carried out to avoid the environmental risks caused by the use of chelators, such as the leaching of metal-chelate complexes in to groundwater (Meers et al., 2005). For example, the formulation of slow-release EDTA granules by coating with silicates (Li et al., 2005), or on the formulation of biodegradable aminopolycarboxylic acids (APCAs) like EDDS, NTA, methylglycinediacetate (MGDA) and hydroxyminodisuccinic acid (HIDS) (Grčman et al., 2003; Tamura et al., 2005; Freitas and Nascimento, 2009; Rahman et al., 2009). EDTA is one of the best complexing agents used for the extraction of trace metals from contaminated soils, since it forms particularly stable and soluble complexes with many metal cations in molar ratio 1: 1 and over a wide range of pH values, but the main problem is the high solubility and mobility of metal complexes. Among organic acids, citric acid has shown high extraction capacity of trace metals (Polettini, 2009) and has a low environmental impact, as it is easily degradable both in aerobic and anaerobic conditions (Romkens, 2002). Other chelating organic substances, at lower cost and biodegradable can be useful for phytoxtraction, including for example pyruvic, citric, succinic, fumaric, malic, oxaloacetic, VFA (formic, acetic, propionic and butyric), oxalic, glycolic, lactic, tartaric, cinnamic, caffeic, ferulic and gallic acid (Bassi et al., 2000; Polettini, 2009; Romkens, 2002).

The mechanism of action of sepiolite and phosphogypsum towards TEs is quite different, and mainly refers to their absorption capacity (Yang et al., 2012). Adsorption is considered one of the most interesting alternative mechanisms for the removal of soluble heavy metals and other ions in heterogeneous systems, for its simplicity of application (Kocaoba et al., 2009) and for the possibility of using natural adsorbents with low cost such as agricultural wastes (wool, rice straw, coconut husks and peat moss), or processing waste biomass (Kocaoba et al., 2009). Other natural materials such as clay and zeolite (Kara et al., 2003) have been found useful adsorbents for heavy metals; in fact, zeolite has a high selectivity and absorption capacity due to its high porosity and sieving properties, and they are widely used as ion exchangers. In general, clay minerals, important natural constituents of soils, filter pollutants from water both through ion exchange and above all adsorption mechanisms, thanks to their high specificity of the surface area, chemical and mechanical stability, stratified structures, high capacity to cation exchange, etc. This convenient method is relatively simple and safe due to the operating conditions (Kocaoba et al., 2009).

6.3. Plant selection

The selection of the plant species to be used for a specific phytoremediation technology is equally crucial for the sucess of the process. The current tendence is to select spontaneous wild plant species with presence in the area to be remediated, so as not to alter the existing balance of the ecosystem with the introduction of exotic species, or to use crop plants for non-food purposes in order to obtain an economical benefit during the remediation programme (Kidd et al., 2015). The design of a phytoremediation intervention must therefore always start from the direct observation of the site, and especially searching for the species present naturally in the site as they are well adapted to climatic and pedological conditions, and have adquired certain characteristics of tolerance. Moreover, the plant selection cannot be separated from the evaluation of the degree and depth of the contamination. For example, the decontamination of superficially polluted soils prefers species with a superficial root system and rich in capillaries, typical of herbaceous crops; while for deeper contamination, species with more developed deep root systems such as tree plants (Gómez et al. 2019) are recomended.

The physiological response of the plants to the contamination and the mechanisms of tolerance must be also taken into account for plant selection. Plant species that have adapted particularly well to living on soils with high metal content are called metallophytes. The plant species can be divided into three general types (Baker, 1981; Figure 2.2):

- Accumulator species, if the TEs are taken up from the soil and concentrated in the aboveground parts of the plants;

- indicator species, if the uptake and transport of TEs from roots to shoots is such that the internal concentration reflects the external concentration;
- excluder species, if the concentrations of TEs in the shoots are low and constant even in the presence of high concentrations in the soil, up to the critical value above which the mechanism breaks down and unlimited transport occurs; these have the tendency to accumulate TEs in the roots and limit transport in the aerial parts (Ali et al., 2013).

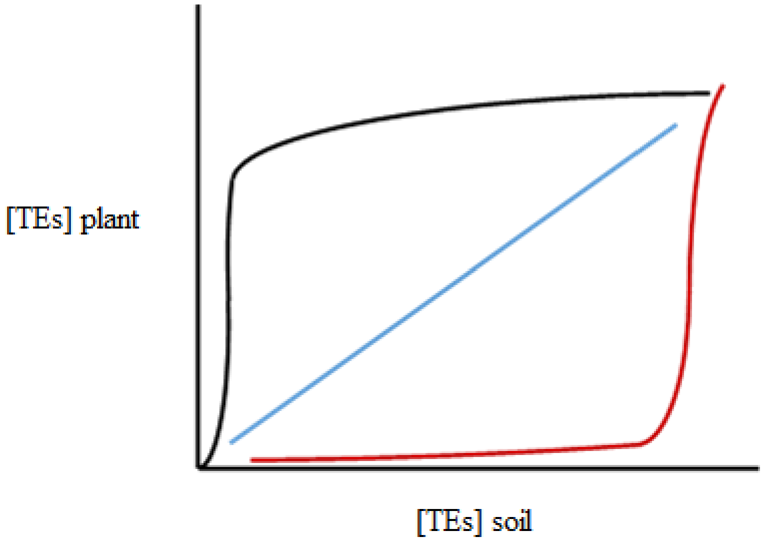

Figure 2.2. Schematic representation of the plant response to trace elements (TEs) in the soil: Accumulator (in black), excluder (in red) and indicator (in blue). Axes are not at real scales.

The ability of plants to extract TEs from the soil and transfer them into their tissues can be assessed through several parameters: the bioconcentration factor (BCF), as the ratio between the total TE concentration in plant tissues (aerial part or roots) and the concentration in the matrix (Conesa et al., 2007), total or soluble (Mench et al., 2010); the accumulation rate (AR), as the total TE content in the tissues during the experimental time (Mench et al., 2010). The translocation ratio (TR; also called translocation factor; Conesa et al., 2007), indicates the ratio between the total concentration of TE present in the aerial parts with that present in the roots (Mench et al., 2010). All these indicators, BCF, AR and TR can also be expressed in percentage terms (Ali et al., 2013).

7. PLANT SPECIES USED FOR THE REMEDIATIONOF CONTAMINATED SOILS AND THEIR CHARACTERISTICS.

The plant species suitable for each phytoremediation technologyshould be tolerant to the contaminant with a specific mechanism to provide the particular physiological characteristics necessary for the efficient accumulation, exclusion, degradation or adsorption of the contaminant.

7.1. Tolerance and mechanisms of tolerance

The tolerance is the ability of the plant to resist high levels of toxic compounds in the growing media. While toxicity is the ability of a potentially toxic substance to produce a plant damage. There are two possibilitiesof tolerance: stress avoidance, which consists on preventing the entrance of the contaminant in the plant tissue; or real mechanisms of tolerance, consisting in fighting the stress caused by the contaminant. When the uptake and translocation of the TEs is favoured, the concentration of TEs in the shoots with respect to roots is > 1. The excluder species maintain the concentration of TEs low in a wide range of soil concentrations until the mechanism of tolerance is surpassed and plants die due to TEs toxicity. The mechanism of tolerance mainly consist of stress avoidance by maintaining the toxic compound at the roots level; the concentration of TEs in shoots with respect to roots is < 1. Indicator plants reflect in their tissue the concentration in the soil; their concentration of TEs in shoots with respect to roots is about 1.

As phytoextraction is based on the uptake of the contaminants by plant biomass and their removal by plant harvesting, the uptake, translocation and yield of the plants condition the success of the process. The amount of metal removed annually per unit area (Q) can be expressed according to the following formula (Magistrelli et al., 2002):

$$Q = C \times B \times n$$

Where ‘C’ is the concentration of the TE in the plant (mg kg^{-1} dw), ‘B’ the biomass produced per crop per unit area (kg m^{-2} dw) and ‘n’ the number of harvests achievable each year.

Consequently, with a view to using plants for phytoextracion, low values of Q often do not allow to obtain an acceptable phytoextraction yield. For phytoextraction to be carried out, plants must be able to accumulate high concentrations of TEs in the removable parts (for example the stems or leaves), and reach a satisfactory quantity of biomass (Kidd et al., 2015). Phytoextraction depends on the annual accumulation of TEs in the aerial biomass, which can then be removed to support soil remediation (Chaney et al., 2007). Some plant species have the ability to accumulate extremely high concentrations of TEs in their above-ground parts under natural conditions without reduction of their biomass and, they are known as hyperaccumulator plants (e.g., TE concentrations in the aerial part oscillate, depending on the element considered, between 100 and 10.000 mg kg^{-1}; Baker and Brooks, 1989).

Several species show TE concentrations in the leaves exceeding the following limits under natural conditions are recognized as hyperaccumulators: 100 for Cd, Se and Tl; 300 for Co, Cr and Cu; 1000 for As, Ni and Pb; 3000 per Zn and 10,000 per Mn (µg g^{-1} dw; Ali et al., 2013; Pollard et al., 2014; Van der Ent et al., 2013). These values are on average 100 times larger than those normally contained in plants growing in metalliferous soils (Ali et al., 2013) and could be further supported by other characteristics such as a BCF> 1, TR> 1, extreme metal tolerance (Van der Ent et al., 2013).

To date, about 400 hyperaccumulatorspecieshave been identified, mostly from the families of *Caryophyllaceae*, *Cyperaceae*, *Poaceae*, *Fabaceae*, *Chenopodiaceae*, *Asteraceae*, but above all the *Brassicaceae* (such as *Thlaspi* now *Noccaea* and *Alyssum*). For instance, *Arabidopsis halleri*can accumulate Cd (>100 mg kg^{-1}) and Zn (>20000 mg kg^{-1}; McGrath et al., 2006) and *Thlaspi (Noccaea) caeraluscens* can accumulate Zn (leaf 100-300 mg kg^{-1}; shoots10.023-25.561mg kg^{-1}; roots 3.307-4.269mg kg^{-1}), Pb (shoots 4.409-4.706 mg kg^{-1}; roots 14.485-51.156 mg kg^{-1}) and Cd (shoots 425-1.006 mg kg^{-1}; roots 174-340 mg kg^{-1}) by their transport through the xylem system (Assunção et al., 2003; El Kheir et al., 2008).

Various studies have shown that the efficiency of phytoextraction can be improved by transferring the genes responsible for hyperaccumulation to species that have a high biomass production (Martínez et al., 2006). The species *Nicotiana glauca*, characterized by a high biomass production, considerable root depth and ease of propagation, was transformed with the wheat PC synthase gene TaPCS1 which conferred increased tolerance to Cd and Pb (longer root length in the transformed plants) and favoured Pb concentration in roots and shoots (Gisbert et al., 2006). The overexpressed gene confers up to 9 and 36 times more Cd and Pb accumulation in the shoots under hydroponic conditions, and a 3- and 6-fold increase in mining soils (Martínez et al., 2006. The modified species showed a greater accumulation of heavy metals and boron, and a yield of biomass 100 times greater in the genetically modified plant (Martínez et al., 2006). In contrast, the species *Thlaspi caerulescens,* whose hyperaccumulation capacity is well known (McGrath et al., 2006), was unable to survive in the soil highly contaminated by Pb and Zn (> 11.000 mg kg^{-1} of Pb and> 4.500 mg kg^{-1} for Zn) (Martínez et al., 2006).

Other option for phytoextraction is to use the so-called “biomass plants”, which are able to accumulate toxic elements in their tissues and, although not been able to hyperaccumulate, they provide a high yield of dry matter. Most of them belong to the *Brassicaceae* family (including *Brassica napus*, *Brassica juncea* L. *Czern* and *Raphanus sativus* L.; Mosca et al., 2004), but other species can be used: *Helianthus annuus* L., *Nicotiana tabacum*, *Linum usitatissimum*, *Mentha* sp., *Gossypium herbaceum*, *Zea mays* (Navari-Izzo et al., 2004), *Lolium multiflorum* L. (Rizzi et al., 2004), *Hordeum vulgare* L. (Calace et al., 2004; Molas and Baran, 2004) and *Medicago sativa* L. (Hernandez-Pinero et al., 2002; Peralta-Videa et al., 2002). In general, species that show a moderate-high bioconcentration factor of metals together with good biomass production can reach a considerable extraction of TEs (Kidd et al., 2015). Plants that produce a high amount of biomass and accumulate moderate levels of TEs in their tissues are recommended for phytoextraction, precisely because the low concentration of contaminants in the tissues can be compensated by the high amount of aerial biomass (Van Oosten and Maggio, 2015).

Such biomass plants can be useful for assited or induced phytoextraction. For example, in *Brassica juncea*, *Helianthus annuus* and *Zea mays* a significant increase in Pb translocation to the harvestable parts of the plant was observed following the addition of K_2EDTA to the medium (Magistrelli et al., 2002). In particular, under these conditions, *B. juncea* showed an accumulation of Pb in the aerial parts with values up to 1.5% of dry biomass (Huang and Cunningham, 1996; Huang et al., 1997; Blaylock et al., 1997). The uptake of the metal-chelate complex is an acute process that takes place in one to two weeks, at the end of which, when obvious symptoms of toxicity appear on the plant, it is collected and disposed of (Magistrelli et al., 2002). Moreover, in assisted phytoextraction, toxicity to the plant often seems to be associated, rather than to the TE, with the excess of chelate added (normally associated with Ca^{2+} deficiency, essential for the thickening and robustness of cell walls, and Mg^{2+} deficiency essential for photosynthesis), which presumably modifies the balance between micro and macro nutrients, regulated with extreme precision in all cells (Clemens, 2001). At the end of a remediation intervention through enhanced phytoextraction, it is important to plan the management of the produced plant biomass, evaluating the most suitable disposal method in relation to the type and concentration of the contaminants present, and to the regulatory requirements on waste.

For phytoimmobilization and phytostabilization, plant species must be tolerant to the toxicity by exclusion. The contaminants remain retainedin the root system and in the rhizosphere (Hunce et al., 2019), with reduced transport from the roots to the shoots. Candidate species to carry out phytostabilization must show themselves as TEs excluders, with a BCF and a TR <1 (Mench et al., 2010; Mendez and Maier, 2008), in order to minimize translocation in the aerial parts and diffusion in the food chain. These plants have the ability to immobilize pollutants in the soil through mechanisms of absorption and accumulation within the roots, adsorption on the root surface, precipitation in the rhizosphere, and physical stabilization of the soil (Suthan Suthersan, 2002).

There are three fundamental processes that characterize the technique of phytostabilization:

1) **Phytostabilization in the root area**: plants release proteins and enzymes into the rhizosphere that cause the precipitation or immobilization of contaminants in the surrounding soil, diminishing the bioavailable fraction of the pollutant. The addition of soil improvers such as phosphates, lime, gypsum and organic matter, can improve the sequestering capacity against certain metals (Pb, Cd, Zn, As). In addition, the presence of mycorrhizae in the rhizosphere, in particular ectomycorrhizae, can increase the immobilization of inorganic contaminants and simultaneously have beneficial effects on plant nutrition (Tsao, 2003).

2) **Phytostatization on root membranes**: proteins and enzymes in the root cell walls are able to bind and stabilize the contaminant on the external surface of the root membranes; in this way, its entry inside the plant is avoided.

3) **Phytostabilization in radical cells**: proteins and enzymes in cell walls can facilitate the transport of contaminants through radical membranes. Once absorbed, they can be retained in the vacuoles of the radical cells, thus avoiding their transfer to aboveground organs (I.T.R.C., 2001).

Plants not only directly stabilize contaminants, but also act indirectly by reducing their spread due to erosive agents such as rain and wind (I.T.R.C., 1999). For the application of this technique, robust trees with an extensive root system (poplars, willows) or herbaceous plants with very dense fibrous roots that prevent soil erosion and retain contaminants are required (Schnoor, 1998). Among herbaceous plants, *Agrostis tenuis* has been found effective for the treatment of soils contaminated by Pb, Zn and Cu, and *Festuca rubra* for Pb and Zn (E.P.A., 2000).

7.2. Examples of phytoextraction and phytostabilization

- ***Arundo donax* L.:** this plant species is characterized by a high tolerance to different TEs (Cd, Ni, Pb, Zn) (Papazoglou et al., 2005; Kos et al., 2003a) and it is particularly suitable for phytoextraction in sites heavily polluted with TEs such as Zn, Pb, Cd and Ni and showed no symptoms of toxicity up to 600 μg L^{-1} of As when grow in wastewater (Mirza et al., 2010; Table 2.1). The characteristics that make it suitable for phytoextraction are: rapid growth, poor crop needs, adaptability to different soil and drought conditions, deep root system and high biomass production (Grippi et al., 2020). The ability to accumulate occurs mainly in the aerial part of the plant (stems and leaves), thus allowing an easy removal from the site (Gou and Miao, 2010). Furthermore, *A. donax* is not eaten by animals and this prevents toxic and persistent substances from entering the food chain.

- ***Cannabis sativa* L.**: This species has been successfully used in the treatment of water and soils contaminated by TEs (Table 2.2.), radionuclides, and aromatic compounds (hydrocarbons and PCBs) (Citterio et al., 2003; Linger et al., 2002; Vandenhove et al.,2005; Kos et al., 2003b, Patent WO/2008/029423). The high biomass production, the large plasticity, which allows hemp to be grown under a wide variety of agro-ecological conditions, and the possibility to use its biomass in non-food industries, make this species attractive for phytoremediation (Linger et al., 2002; Citterio et al., 2003; Arru et al., 2004). Excellent phytoextraction capacities are attributed to the hemp both of organic contaminants and TEs (in particular Pb, Cd, Cr, Hg, Zn and radioactive elements) accumulated in a variable percentage depending on the element and the vegetative organ.

- ***Cynara cardunculus spp. L.:*** It can be cultivated as a biomass crop in rural and marginal environments due to its reduced needs and perfect adaptation to Mediterranean environments. Many studies have highlighted the ability of the variety C. *cardunculus sylvestris*, or wild thistle, to accumulate several TEs such as Pb, Zn, Cd, As (Dominguez et al., 2017; Table 2.3).

- ***Dittricha viscosa***: this plant species produced a high biomass with high calorific value in highly degraded soils (Dominguez et al., 2017; Table 2.4).

- ***Festuca arundinacea* S.:** Its developed root system is effective for reducing the leaching of pollutants and soil erosion in general, and shows an excellent phytostabilization capacity. Studies have shown high levels of accumulation of Pb (21.500 mg kg^{-1}) and Zn (3.000 mg kg^{-1}) especially in roots (Begonia et al., 2005; Table 2.5). This plant can be used for the remediation of soils contaminated by heavy metals through phytostabilization (Begonia et al., 2005; Soleimani and Hajabbasi, 2010).

- ***Helianthus annuus* L.:** the high biomass production rate, the vigorous transpiration potential and the dense root system make sunflower useful plant for phytoremediation projects. It can extract pollutants from soil, such as Pb, Cd, Cr, Ni (Chen and Cutright, 2001) and radionuclides (Dushenkov et al., 1999; Table 2.6). *H. annuus* can also grown to remove toxic metals and uranium from aqueous solutions by rhizofiltration (Dushenkov et al., 1995).

- ***Jatropha curcas:*** it has excellent abilities towards phytoremediation of heavy metals, such as Al, Fe, Cr, Mn, As, Zn, Cd and Pb (Table 2.7), but also towards hydrocarbons and pesticides soil remediation (Yadav et al., 2009; Kumar et al., 2008).

- ***Miscanthus sp.:*** the accumulation of heavy metals, such as As, Sn, Cd, Cr, Cu, Ni, Pb, Zn and Al, occurs mainly in below ground (brooches and rhizomes) while minimum in above ground (stems and leaves) biomass (Pandey et al. 2016; Nsanganwimana et al., 2014). Moreover, Miscanthus, in particular *M. giganteus*, is useful in the biodegradation of organic pollutants such as polycyclic aromatic hydrocarbons, petroleum and pesticides by its root exudates (Técher et al., 2011). Based on Miscanthus' capacity to sequestrate inorganic contaminants into the root system and to induce dissipation of persistent organic contaminants in soil, these plant species are favorable for phytostabilization and phytodegradation (Table 2.8).

- ***Silybum marianum:*** It is able to colonize highly contaminated soils; biomass production and calorific value are not affected by the presence of high concentrations of TEs (Dominguez et al., 2017; Clemente et al., 2019; Table 2.9).

Table 2.1. Case studies of TEs phytoremediation usning *A. donax* L.

Contaminants	**Phytotechnology**	**Contaminant concn.**	**Plant effects**	**Others**	**Reference**
Arsenic	Phytoextraction in wastewater	As_2O_3 (0, 50, 100, 300, 600 and 1000 µg L^{-1} As)	Increasing As concentration (50 to 600 µg L^{-1}) in growing media increased shoot and root biomass without toxicity. Elevated oxidative stress at 1000 µg L^{-1} of As.		Mirza et al. (2010)
Pb, Zn and Cd	Induced soil phytoextraction	Pb: 1100 mg kg^{-1};Zn: 800 mg kg^{-1}; Cd: 5.5 mg kg^{-1}	With EDTA: Pb: 26.95 mg kg^{-1};Zn: 71.44 mg kg^{-1}; Cd:4.02 mg kg^{-1}	Phytoextraction potential (kg ha^{-1}): 0.54 of Pb, 1.43 of Zn, 0.08 of Cd.	Grčman and Lestan. (2003)
Cd and Ni	Soil phytoextraction	Metals added through irrigation: Treatment 5 ppm: 0.085 mM Ni, 0.05 mM Cd Treatment 500 ppm: 0.85mM Ni, 0.24mM Cd; Treatment 100ppm: 1.7mM Ni, 0.48mM Cd	Plants in all treatments showed no detrimental or toxic symptoms and increased in stem height, diameter, and number of nodes, and similar plant production with respect to treatment without metals.	Photosynthetic rates of the plants were unafected by the treaments.	Papazoglou et al. (2005)

Table 2.2. Case studies of TEs phytoremediation using *C. sativa* L.

Contaminants	**Phytotechnology**	**Contaminant conc.**	**Plant effects**	**Others**	**Reference**
Cd, Cr and Ni	Phytoaccululation for Cd and Ni. Tolerant plant for Cr: Phytostabilization	Soil 1: 27 µg g^{-1} Cd, 74 µg g^{-1} Ni, 126 µg g^{-1} Cr. Soil 2: 82 µg g^{-1} Cd, 115 µg g^{-1} Ni, 139 µg g^{-1} Cr.	No significant alteration in plant growth or morphology was detected. A high hemp reactivity to heavy metal stress with an increase in phytochelatin and DNA content.	Metals were preferentially accumulated in the roots and only partially translocated to the above-ground tissues.	Citterio et al. (2003)
Pb, Zn and Cd	Induced phytoextraction.	Addition of 5 mmol kg^{-1} EDDS: Pb uptake: 220.6 mg kg^{-1}; Zn uptake: 105.1 mg kg^{-1}; Cd uptake: 0.76 mg kg^{-1}. Addition of 10 mmol/kg of EDDS: Pb uptake: 1053 mg kg^{-1}; Zn uptake: 211mg kg^{-1}; Cd uptake: 5.4 mg kg^{-1}	The percentage of Pb phytoextracted in a single cycle was only approx. 0.6% of the total Pb present in the upper 30 cm of soil. The achieved Pb concentration in *C. sativa* was far from the Pb concentrations required for efficient soil remediation within a reasonable time span.	Phytoextraction potential: 5.51 kg ha^{-1} Pb; 263 kg ha^{-1} Zn; 0,019 kg ha^{-1} Cd.	Kos et al. (2003b)

Table 2.3. Case studies of TEs phytoremediation usning *C. cardunculus* L.

Contaminants	Phytotechnology	Contaminant conc.	Plant effects	Others	Reference
As, Cd, Cu, Fe, Pb, Zn	Phytoextraction	Pseudo total TE in soil (mg kg^{-1}): $3.6<As<136.6$ $0.09<Cd<1.59$ $23.2<Cu<159.0$ $2.282<Fe<8364$ $11.6<Pb<189.8$ $15.6<Zn<484.3$	High capacity for Cu uptake from soil and for its translocation and accumulation into aboveground biomass.	TE concentrations in plant shoots (mg kg^{-1}): $0.08<As<0.49$ $0.10<Cd<1.59$ $60.6<Cu<87.6$ $47.9<Zn<148.5$	Dominguez et al. (2017)

Table 2.4. Case studies of TEs phytoremediation usning *D. viscosa* L.

Contaminants	Phytotechnology	Contaminant conc.	Plant effects	Others	Reference
As, Cd, Cu, Fe, Pb, Zn	Phytoextraction or phytostabilization	Pseudo total TE in soil (mg kg^{-1}): $3.6<As<136.6$ $0.09<Cd<1.59$ $23.2<Cu<159.0$ $2.282<Fe<8364$ $11.6<Pb<189.8$ $15.6<Zn<484.3$	High biomass yield in soils with high concentrations of soluble TE. Given the high calorific value of its biomass and the large extension of its root system this species could be considered a bioenergy crop.	TE concentrations in plant shoots (mg kg^{-1}): $0.28<As<0.61$ $0.28<Cd<3.16$ $47.0<Cu<105.2$ $44.6<Zn<123.8$	Dominguez et al. (2017)

Table 2.5. Case studies of TEs phytoremediation usning *F. arundinacea* S.

Contaminants	**Phytotechnology**	**Contaminant conc.**	**Plant effects**	**Others**	**Reference**
Pb	Induced phytoextraction	0, 1000, 2000 mg Pb kg^{-1}dry soil.	High plant biomass, tolerate high levels of Pb and chelates applied to the soil. No phytotoxic effects of Pb and/or chelates except for a slight reduction of radical biomass.	Six weeks after planting, aqueous solutions of EDTA (0, 5 mmol kg^{-1} dry soil) and acetic acid (5 mmol kg^{-1} dry soil) were applied to the root zone, and all plants were harvested a week later.	Begonia et al. (2005)

Table 2.6. Case studies of TEs phytoremediation usning *H. annuus* L.

Contaminants	**Phytotechnology**	**Contaminant conc.**	**Plant effects**	**Others**	**Reference**
^{137}Cs	Phytostabilisation	Total ^{137}Cs removed from soil by plant was 319 Bq m^{-2}	The roots had a greater ^{137}Cs activity compared to the above ground parts. The bioaccumulation coefficient was about 0.25.		Dushenkov et al. (1999)
As, Cu, Fe, Pb, Zn	Phytostabilisation	Cu 230 μg g^{-1}, Pb 19129 μg g^{-1} Zn 2257 μg g^{-1} As 1976 μg g^{-1} Fe 215 mg g^{-1}.	TE transfer to the aerial parts is limited, which makes biomass recycling possible, with low environmental concern.		Bernal et al. (2019)

Table 2.7. Case studies of TEs phytoremediation usning *J. curcas* L.

Contaminants	Phytotechnology	Contaminant conc.	Plant effects	Others	Reference
As, Cr, Zn	Phytostabilisation	0,25,50,100,250 and 500 mg kg^{-1} for As and Cr, 0, 500, 1000, 2000, 3000 and 4000 mg kg^{-1} for Zn.	Concentration in roots (mg kg^{-1}): $2.8<As<29.0$ $1.5<Cr<8.5$ $4.3<As<674.9$ Concentration in shoot (mg kg^{-1}): $0.4<As<12.5$ $0.2<Cr<3.6$ $0.3<Zn<120.9$	Plants survived on As, Cr and Zn contaminated soils up to 250, 100 and 3000 mg kg^{-1}, respectively	Yadav et al. (2009)

Table 2.8. Case studies of TEs phytoremediation usning *Miscanthus sp.* plants.

Contaminants	Phytotechnology	Contaminant conc.	Plant effects	Others	Reference
As, Cd, Cu, Cr, Pb, Zn, Xenobiotics.	Phytostabilisation, Phytodegradation	Soil pH 8.5 = 78 mg kg^{-1} As Soil pH 5.2= 13.7 mg kg^{-1} Cd Soil pH 6.8 = 130 mg kg^{-1} Cu, 271 mg kg^{-1} Pb, 365 mg kg^{-1} Zn, 55 mg kg^{-1} Cr	TE accumulation in roots, and limited TE transfer to shoots, promoted degradation of organic xenobiotics and improved soil quality. *Miscanthus* is suitable crop for combining biomass production and ecological restoration of contaminated and marginal land.	*M. giganteus* reduce human and environmental risks; a suitable option for the phytomanagement of contaminated sites.	Nsanganwimana et al. (2014)

Table 2.9. Case studies of TEs phytoremediation usning *S. marianum* L.

Contaminants	**Phytotechnology**	**Contaminant conc.**	**Plant effects**	**Others**	**Reference**
As, Cd, Cu, Fe, Pb, Zn	Phytoextraction	Pseudo total TE in soil (mg kg^{-1}): $3.6<As<136.6$ $0.09<Cd<1.59$ $23.2<Cu<159.0$ $2.282<Fe<8364$ $11.6<Pb<189.8$ $15.6<Zn<484.3$	The results suggest a significant accumulation of TE in above-ground biomass in *S. marianum*.	TE concentrations in plant shoots (mg kg^{-1}): $0.20<As<1.45$ $0.16<Cd<2.72$ $51.7<Cu<69.8$ $43.9<Zn<359.7$	Dominguez et al. (2017)
As, Cd, Cu, Mn, Pb, Zn	Phytostabilisation	Pseudo total TE in soil (mg kg^{-1}): As 1976, Cd 12, Cu 230, Mn 968, Pb 19, Zn 2257.	Plant survival in heavily contaminated soils. The use of native plants in combination with the amendments reduced soluble, mobile and available TEs forms in the soil. The TE concentrations in the aerial part of the plants were considered normal and do not represent any environmental concern.	Amendments used: solid fraction of pig slurry; paper mill sludge; a commercial red mud derivative.	Clemente et al. (2019)

8. PLANT BIOMASS FROM PHYTOREMEDIATION AS A SOURCE OF BIOENERGY

One of the major dilemmas that the phytoremediation technologies has tocope with is the use of the plant biomass derived from the restoration of contaminated soils (Pandey et al., 2016). The goal to achieve is to recycle these biomasses in a clean way, obtaining useful derived products and materials, from bio-plastics to dyes, from the transformation of fibers to the production of materials for bio-construction and the production of biofuels. This would make the whole process economically sustainable and would give an even more "noble" purpose to the phytoremediation intervention (Pandey et al., 2016). Recently, the use of biomass has mainly aimed at the production of biofuels (Gelleti et al., 2006; Cencič et al., 2007), which could replace fosil fuels derived from oil, representing an environmental friendly option.

Valid options for the recycling of plant biomass produced during the phytoremediation of contaminated land include the production of renewable energy, such as biogas or thermal energy (Iaboni and De Stefanis, 2003; Grippi et al., 2020). The presence of TEs and the nature of the organic matter can affect the biodegradability of biomass (Bernal et al., 2019). One of the major challenges in the use of biomass for energy production is the transfer of pollutants (such as TE) to plants (Van Ginneken et al.,2007; Gomes, 2012; Sas-Nowosielska et al., 2004), affecting the bioenergetic value. In the case of species used for phytotabilisation, the transfer of TEs to the aereal parts is limited, which makes their recycling possible, with little environmental concern.

Table 2.10 shows the activation Energy (Ea), Gross calorific and thermal decomposition values of the main bioenergy crops useful for phytoremediation. The main technologies for the thermochemical conversion of biomass are combustion, gasification and pyrolysis (McKendry, 2002). Pyrolysis has some advantages over combustion and gasification: it requires relatively lower temperatures, no oxygen and a liquid bio-oil can be produced (syngas) (Bridgwater et al., 2001). Thermogravimetric analysis (TGA) is frequently applied to study the pyrolytic kinetics of lignocellulosic biomass (Chen et al., 2017; Damartzis et al., 2011; Ferreira et al., 2016). The analysis of the gases produced during pyrolysis is, however, important for a full characterization of the environmental implications of this conversion technology and the potential production of syngas.

Thermal analysis and differential scanning calorimetry (DSC) demonstrated their usefullness as techniques both for the evaluation of the thermal characteristics of different organic materials and for the determination of their most adequate degradation process to obtain energy. In fact, both thermogravimetric analysis (TGA) and derivative thermogravimetric curves (DTG) are useful instruments for the evaluation of the behaviour of a material when subjected to temperature changes in combustion or pyrolysis processes (Bernal et al., 2019). Several studies have assessed the possible influence of high concentrations of soluble TEs in the growth medium on the quality of biomass for energy production through pyrolysis (Dominguez et al., 2017). Soil contamination could affect the chemical composition of plants that lead to lower biomass quality for biofuel production (Madejón et al., 2016).

Soil pollution inevitably leads to changes in the physical and chemical properties of biomass, properties on which their use for energy production depends (Grippi et al., 2020): combustion requires a low moisture and ash content; the production of biogas through anaerobic digestion (AD) requires a high content of biodegradable organic matter; the production of ethanol requires high amounts of sugars and cellulosic components.

The analysis of the biomass behaviour during thermochemical conversion is essential for a full identification of their potential as bioenergy crops. The main transformation of biomass takes place at low temperatures (150-380 °C), and lower temperature values were found for *C. sativa* (225-375 °C) and *A. donax* (180-370 °C), than for *C. zizanoides* L. (200-450 °C) and *R. communis* (160-520 °C) indicating the presence of readily-available organic substances (Table 2.10).The activation energy values were all between 110 and 400 kJ mol^{-1} except for *H. annuus* (78 kJ mol^{-1}) and *J. curcas* (40 kJ mol^{-1}); the gross calorific values were in the range 16-19 MJ kg^{-1} for all plant biomass tested (Table 2.10); being *C. carduncul*us and *R. communis* the less suitable for thermal energy production due to their low calorific values and high temperature for thermal decomposition. The values found are not far from the average GCV values of commonly used fuels, such as coal (18 MJ kg^{-1}), while they are slightly lower than petroleum-derived fuels with HHV values ranging from about 25 MJ kg^{-1} (for alcohols) to 55 MJ kg^{-1} (for methane) (Dincer and Zamfirescu, 2014). Table 2.11 shows the possible uses of the plant biomass from phytoremediation experiments for the production of biofuels (Pandey et al., 2016).

Table 2.10. Bioenergenic characteristic of crops used in phytoremediation. Activation energy: Ea, by α=0.5 FWO method.

Crops (Families)	**Gross calorific value (MJ kg^{-1})**	**Ea (KJ mol^{-1})**	**Thermal decomposition (°C)**	**References**
***Arundo donax* L. (Poaceae)**	17	110	1) 180-370 2) 370-750	Jeguirim et al. (2010)
***Brassica* spp (Brassicaceae)**	19	150-404	200-450	Várhegyiet al. (2009) Karaosmanoglu et al. (2001) Licata et al. (2017)
***Cannabis sativa* L. (Cannabaceae)**	17-19	60-174	1) 225-325 325-375 2) 250-500	Kolarikova et al. (2013) Branca et al. (2016)
***Chrysopogon zizanoides* L. (Poaceae)**	16	150-189	1) 30-200 2) 200-450 3) 450-800	Thakur et al. (2018)
***Cynara cardunculus spp.* (Compositae)**	15	38-356	200-500	Damartis et al. (2011)
***Dittricha viscosa*L. (Asteraceae)**	-	317	1) 60-180 2) 180-570 (200-400 250–380) 3) 570-800	Dominguez et al. (2017)
***Helianthus annus* L. (Asteraceae)**	17-21	78	353-542	Hunce et al. (2019) Zabaniotou et al. (2008) Nehdi et al. (2013)
***Jatropha curcas* L. (Euphorbiaceae)**	18-39	40	113-318	Ružbarský et al. (2014) Jain et al. (2012)
***Miscanthus sp.* (Poaceae)**	18	128	250-400	Dominguez et al. (2017) Cortés et al. (2015)
***Ricinus communis* L. (Euphorbiaceae)**	14	216	1) 128-160 2) 160-520 3) 150-900	Kaur et al. (2018)
***Silybum marianum* L. (Compositae)**	14-17	295	1) 60-180 2) 180-570 (200-400 250-380) 3) 570-800	Hunce et al. (2019) Dominguez et al. (2017)

*= Flynn-Wall-Ozawa: This method covers the determination of the overall kinetic parameters for exothermic reactions.

Table 2.11. Multipurpose benefits of phytoremediation crops according to the pollutant.

Plants (Families)	Pollutants	Trace element in biomass (mg kg^{-1})	Potential Phytoproducts	References
***Arundo donax* L. (Poaceae)**	Nutrients, TEs (Cd, As, Ni, Pb), POPs.		Bioethanol from biomass, charchoal	Pandey et al. (2016) Liu et al. (2012)
***Brassica spp* (Brassicaceae)**	TEs (Cd, Cu, Ni, Zn, Cr, Se, Pb), POPs	Pb 10-106	Bionergy production from biomass	Pandey et al. (2016)
***Cannabis sativa* L. (Cannabaceae)**	TEs (Cd, Hg, Zn, Cr, Pb)	-	Bionergy production from biomass	Pandey et al. (2016)
***Chrysopogon zizanoides* L. (Poaceae)**	TEs (Al, As, Mn, Cd, Ni, Cu, Se, Hg, Zn, Cr, Pb), Pesticides(Diuron, Atrazine), DDT	-	Bioethanol from biomass	Truonge Baker (1998)
***Cynara cardunculus spp.* (Compositae)**	TEs (Cd, As, Zn, Pb)	As 0-1 Cd 0.1-1.8 Cu 29-146 Zn 32-239	Bionergy production from biomass	Dominguez at al. (2017) Pandey et al. (2016)
***Dittricha viscosa* L. (Asteraceae)**	TEs (Cd, As, Zn, Pb)	As 0.1-1.1 Cd 0.2-3.3 Cu 33-146 Zn 31-138	Bionergy production from biomass	Dominguez et al. (2017)
***Festuca arundinacea* S. (Poaceae)**	TEs (Cd, Zn, Pb), PCB	Pb 21.500 (roots) Zn 3000	Bioethanol from biomass	Begonia et al. (2005)
***Helianthus annus*L. (Asteraceae)**	POPs, TEs (Zn, Cu, Cd, Cr, Pb, Hg), Radionuclides (Sr, Ce134 &137, U)	-	Bioenergy, bioethanol, charcoal	Smith et al. (2013) Cutright et al. (2010) Dushenkov et al. (1999) McIntyre (2003)
***Jatropha curcas* L. (Euphorbiaceae)**	POPs, TEs (Al, Fe, Cr, Mn, Ar, Zn, Cd, Pb), pesticides	-	Biodiesel from seed oil, biofertilizer, charcoal	Pandey et al. (2016) Abhilash et al. (2013)
***Miscanthus sp* (Poaceae)**	TEs (Al, As, Sn, Cu, Cr, Ni, Zn, Cd, Pb), POPs, pesticides	-	Bioethanol from biomass, biogas	StClair et al. (2008) Zhao et al. (2012) Pandey et al. (2016) Técher et al. (2011)
***Ricinus communis* L. (Euphorbiaceae)**	TEs(Cd, Pb, Zn, As), DDT	Cd 400	Bioenergy crop, biodiesel	Huang et al. (2011) Costa et al. (2012) Pandey et al. (2016)

			production from seed oil	
***Silybum marianum* L. (Compositae)**	TEs (Cd)	As 0-2 Cd 0.15-2.92 Cu 40-83 Zn 36.4-430.6	Bionergy production from biomass	Dominguez et al. (2017)

Bernal et al. (2019) suggested that the properties of the plant biomass (as thermochemical conversion, activation energy and gross calorific value), for their pyrolysis, were not significantly affected by the presence of high concentrations of soluble TEs in the soil. To confirm the suitability of the biomass grown in TE contaminated soils in the Mediterranean region for bioenergy purposes, other data should be considered in future studies, such as a complete characterization of the energy balance from crop settlement to biomass processing, as well as an assessment of potential problems associated with the production of metal-containing ash during thermochemical conversion.

Therefore, there is certainly a potential link between the cultivation of energy crops and the phytoremediation of contaminated soils (Pidlisnyuk et al., 2014). More attention has been paid to fast-growing wood species, less to non-woody perennial crops (Técher et al., 2011) and only a few sources are found on perennial herbaceous crops (Rakhmetov et al., 2007). Also, the introduction of new crops in any ecosystem leads to new interrelations between vegetation, soil, water and air. In addition, the use of native plants would allow a higher chance of survival, growth and reproduction under these environmental stress conditions than plants introduced from other environments (Adriano, 2001; Antonsiewicz et al., 2008).

9. CONCLUSIONS

Phytoremediation is a remediation technology for application on large areas, with low expenses in terms of intervention costs. The recovery of the environmental value of contaminated areas, and its high public acceptance constitute the strengths of this technology. Furthermore, from the studies on phytoremediation, new tendences have emerged. The vegetative cover of an already reclaimed site has the dual effect of physical stabilization of the soil (by controlling erosive processes) and refinement of the remediation process (through degradation mechanisms, accumulation and immobilization of residual contaminants). Also, phytoremediation can be improved by the use of herbaceous species specific to the type of contamination and site, and in particular the use of native plants that have a greater chance of survival, growth and reproduction in conditions of a particular environmental stress due to the specific soil contaminants. The most innovative perspective of phytoremediation consists on the use of plant biomass for the bioenergy production, through low-cost techniques. Special interest for the combination of phytoremediation and bioenergy (thermal) production has been found for *Arundo donax, Cannabis sativa, Helianthus annuus, Jatropha curcas* and *Miscanthus*. The use of other species, like *Silybum marianum,* for biogas production has also be envisaged. Therefore, the use of high biomass energy crops opens a new perspective for developing a low cost and environmentally friendly phytoremediation technology applicable to different contaminated soils under diverse climatic and environmental conditions. Research in this area gives hope that in the near future phytoremediation will occupy a prominent place among remediation technologies.

References

Abdel-Shafy, H.I. Mansour, M.S.M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum, 25*(1), 107–123. https://doi.org/10.1016/j.ejpe.2015.03.011

Abhilash, P.C., Singh, B., Srivastava, P., Schaeffer, A., Singh, N. (2013). Remediation of lindane by *Jatropha curcas* L: utilization of multipurpose species for rhizoremediation. *Biomass Bioenergy, 51,*189–93.

Acosta, J.A., Jansen, B., Kalbitz, K., Faz, A., Martínez-Martínez, S., (2011). Salinity increases mobility of heavy metals in soils. *Chemosphere, 85*(8), 1318-1324.

Adriano, D.C. (2001). *Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals,* pp. 2-13. New York: Springer-Verlag.

Ali, H., Khan, E., Sajad, M.A. (2013). Phytoremediation of heavy metals - Concepts and applications. *Chemosphere, 91,* 869 - 881.

Alloway, B.J. (2013). Introduction. *Heavy Metals in Soils* (III ed.), pp. 3-10. Dordrecht: Springer.

Amer, N., Al Chami, Z., Al Bitar, L., Mondelli, D., Dumontet, S. (2013). Evaluation of *Atriplex halimus*, *Medicago lupulina* and *Portulaca oleracea* for phytoremediation of Ni, Pb and Zn. *International Journal of Phytoremediation, 15* (5), 498 - 512.

Agenzia Nazionale per la Protezione dell'Ambiente (ANPA). (2001). Atlante degli indicatori del suolo. RTI CTN_SSC 3/2001. Roma. Available at http://ctntes.arpa.piemonte.it/pubblicazioni/RTI_CTN_SSC_3_2001_1_.pdf (accessed November 2020).

Antonsiewicz, D.M., Escude-Duran, C., Wierzbowska, E., Sklodowska, A. (2008). Indigenous plant species with potential for the phytoremediation of arsenic and metal contaminated soil. *Water Air Soil Pollution. 19*, 197-210.

Arru, L., Rognoni, S., Baroncini, M., Bonatti, P.M., Perata, P. (2004). Copper localization in *Cannabis sativa* L. grown in a copper-rich solution. *Euphytica*, *140*, 33–8.

Ashraf, M.A., Maah, M.J., Yusoff, I. (2014). Soil contamination, risk assessment and remediation. In: M.C. Hernandez-Soriano, *Environmental Risk Assessment of Soil Contamination*. Intechopen, 3-56. http://dx.doi.org/10.5772/57287.

Assunção, A.G.L., Schat, H., Aarts, M.G.M. (2003) *Thlaspi caerulescens*, an attractive model species to study heavy metal hyperaccumulation in plants. *New Phytologist*, *159*, 351–360

Audet, P., and Charest, C. (2009). Contribution of arbuscular mycorrhizal simbiosi to in vitro root metal uptake: from trace to toxic metal conditions. *Botany, 87*(10), 913-921. https://doi.org/10.1139/B09-062.

Azanu, D., Mortey, C., Darko, G., Weisser, J.J., Styrishave, B. Abaidoo, R.C. (2016). Uptake of antibiotics from irrigation water by plants. *Chemosphere*, *157,* 107–114. https://doi. org/10.1016/j.chemosphere.2016.05.035

Baker, A. J. M. (1981). Accumulators and excluders - strategies in the response of plants to heavy metals. *Journal of Plant Nutrition*, *3*(1-4), 643-654, https://doi.org/ 10.1080/01904168109362867.

Baker, A.J.M., Brooks, R.R. (1989). Terrestrial higher plants which hyperaccumulate metals. *Biorecovery*, *1*, 81-126.

Barbafieri, M. (2005) . Phytoremediaton in *Bonifica dei siti contaminati*, Milan, Italy: McGraw – Hill.

Bassi, R., Prasher, S. O., & Simpson, B. K. (2000). Extraction of metals from a contaminated sandy soil using citric acid. *Environmental Progress*, *19*(4), 275–282. https://doi.org/10.1002/ep.670190415.

Begonia, M. T., Begonia, G. B., Ighoavodha, M., Gilliard, D. (2005). Lead accumulation by tall fescue (*Festuca arundinacea* schreb.) grown on a lead-contaminated soil. *International Journal of Environmental Research and Public Health*, *2*(2), 228–233. https://doi.org/10.3390/ijerph2005020005

Bernal, M. P., Clemente, R., Walker, D. J. (2009). Interactions of heavy metals with soil organic matter in relation to phytoremediation. In: *Phytoremediation: the Green Salvation of the World* Navarro-Aviño, J.P. (Ed.), pp. 109–129. Kerala, India: Research Signpost.

Bernal, M. P., Gómez, X., Chang, R., Arco-Lázaro, E., Clemente, R. (2019). Strategies for the use of plant biomass obtained in the phytostabilisation of trace-element-contaminated soils. *Biomass and Bioenergy, 126,* 220–230. https://doi.org/10.1016/j.biombioe.2019.05.017

Blaylock, M.J., Salt, D.E, Dushenkov, V., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, Y., (1997), Enhanced accumulation of Pb in indian mustard by soil- applied chelating agents. *Environmental Science and Technology, 31,* 860-865.

Bouchez, T., Blieux, A.L., Dequiedt, S., Domaizon, I., Dufresne, A., Ferreira, S., Godon, J.J., Hellal, J., Joulian, C., Quaiser, A., Martin-Laurent, F., Mauffret, A., Monier, J.M.,Peyret, P., Schmitt-Koplin, P., Sibourg, O., D'oiron, E., Bispo, A., Deportes, I., Grand, C., Cuny, P., Maron, P.A., Ranjard, L. (2016). Molecular microbiology methods for environmental diagnosis. *Environmental Chemistry Letters, 14,* 423–441.

Boxall, A.B.A., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J.P., Verslycke, T., Ankley, G.T., Beazley, K.F., Belanger, S.E., Berninger, J.P., Carriquiriborde, P., Coors, A., DeLeo, P.C., Dyer, S.D., Ericson, J.F., Gagné, F., Giesy, J.P., Gouin, T., Hallstrom, L., Karlsson, M.V., Larsson, D.G.J., Lazorchak, J.M., Mastrocco, F., McLaughlin, A., McMaster, M.E., Meyerhoff, R.D., Moore, R., Parrott, J.L., Snape, J.R., Murray- Smith, R., Servos, M.R., Sibley, P.K., Straub, J.O., Szabo, N.D., Topp, E., Tetreault, G.R., Trudeau, V.L. & Van Der Kraak, G. (2012). Pharmaceuticals and Personal Care Products in the Environment: What Are the Big Questions?.*Environmental Health Perspectives, 120*(9), 1221–1229. https://doi.org/10.1289/ehp.1104477.

Branca, C., Di Blasi, C. (2016). A summative model for the pyrolysis reaction heats of beech wood.*Thermochimica Acta, 638,* 10–16. https://doi.org/10.1016/j.tca.2016.06.006

Bridgwater, A.V., Czernik, S., Piskorz, J. (2001). An overview of fast pyrolysis. In: *Progress in Thermochemical Biomass Conversion*. Bridgwater, A.V. (Ed.), pp. 977. Oxford: Blackwell Science Ltd.

Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., De Deyn, G., de Goede, R., Brussaard, L. (2018). Soil quality – A critical review. *Soil Biology and Biochemistry, 120,* 105-125. https://doi.org/10.1016/j.soilbio.2018.01.030.

Calace, N., Petronio, B.M., Pietroletti, M., Deriu, D., Pompili, L. (2004). Treatment of heavy metal contaminated soli to reduce metal uptake by cultivation. *Recent Research Developments in Agronomy and Horticolture, 1,* 29-46.

Callahan, B., Proctor, D., Relman, D., Fukuyama, J., Holmes, S. (2016). Reproducible research workflow in r for the analysis of personalized human microbiome data. *Pacific Symposium on Biocomputing, 21,* 183–194.

Cenciˇc, V.; Jodice, R.; Migliardi, D.; Picco, D.; Pin, M.; Tomasinsig, E.; Zanotto, G.; Radu, R.; Taccani, R.; Chindaruksa, S. (2007). *I Biocarburanti. Le Filiere Produttive, le Tecnologie, i Vantaggi Ambientali e le Prospettive di Diffusione,* Vol. 27, pp. 26–158. Padriciano, Italy: Area Science Park.

Chalew, T.E.A. & Halden, R.U. (2009). Environmental Exposure of Aquatic and Terrestrial Biota to Triclosan and Triclocarban. *JAWRA Journal of the American Water Resources Association, 45*(1), 4–13. https://doi.org/10.1111/j.1752-1688.2008.00284.x

Chaney, R.L., Malik, M., Li, Y.M., Brown, S.L., Brewew, E.P., Angle, J.S., Baker, A.J.M. (1997). Phytoremediation of soil metals. *Current opinion in Biotechnology, 8,* 279-284.

Chaney, R.L., Y.-M. Li, J.S. Angle, A.J.M. Baker, R.D. Reeves, S.L. Brown, F.A. Homer, M. Malik, and M. Chin. (2000). Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems: Approaches and progress. In: *Phytoremediation of contaminated soil and water*. N. Terry and G.S. Bañuelos (ed.), pp. 131–160. Boca Raton, FL : CRC Press,.

Chaney, R.L., Scott, Angle, J., Broadhurst, C.L., Peters, C.A., Tappero, R.V., Sparks, D.L. (2007). Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. *Journal of Environmental Quality, 36,* 1429 - 1443.

Chen, H., Cutright, T. (2001). EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. *Chemosphere. 45*(1*),* 8-21. https://doi.org.10.1016/s0045-6535(01)00031-5. PMID: 11572587.

Chen, C., Miao, W., Zhou, C., Wu, H. (2017). Thermogravimetric pyrolysis kinetics of bamboo waste via Asymmetric Double Sigmoidal (Asym2sig) function deconvolution. *Bioresource Technology, 225,* 48-57.

Citterio, S., Santagostino, A., Fumagalli, P., Prato, N., Ranalli, P., Sgorbati, S. (2003). Heavy metal tolerance and accumulation of Cd, Cr and Ni by *Cannabis sativa* L. *Plant and Soil, 256*(2), 243 - 252.

Clemens, S. (2001). Molecular mechanisms of plant metal tolerance and homeostasis. *Planta, 212*, 475-486.

Clemens, S., Palmgren, M.G., Krämer, U. (2002). A long way ahead: understanding and engineering plant metal accumulation. *Trend in Plant Science, 7*(7), 309-315. https://doi.org.10.1016/s1360-1385(02)02295-1.

Clemens, S. (2006). Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie, 88,* 1707-1719.

Clemente, R., Pardo, T., Madejón, P., Madejón, E., Bernal, M.P. (2015). Food byproducts as amendments in trace elements contaminated soils. *Food Research Internastional Journal, 73,* 176-189. https://doi.org/10.1016/j.foodres.2015.03.040.

Clemente, R., Arco-Lázaro, E., Pardo, T., Martín, I., Sánchez-Guerrero, A., Sevilla, F., Bernal, M. P. (2019). Combination of soil organic and inorganic amendments helps plants overcome trace element induced oxidative stress and allows phytostabilisation. *Chemosphere, 223*, 223–231. https://doi.org/10.1016/j.chemosphere.2019.02.056.

Clemente, R., Sáez-Tovar, J.A., Bernal, M.P. (2020). Extractability, distribution among different particle size fractions, and phytotoxicity of Cu and Zn in composts made with the separated solid fraction of pig slurry. *Frontiers in Sustainable Food Systems, 4*, 1-8. https://doi.org. 10.3389/fsufs.2020.00002.

Collins, R.N., Merrington, G., McLaughlin, M.J., Knudsen, C. (2002). Uptake of intact zinc- ethylenediaminetetraacetic acid from soil is dependent on plant species and complex concentration. *Environmental Toxicology and Chemistry, 21*, 1940- 1945.

Committee on Bioavailability of Contaminants in Soils and Sediments. (2002). Bioavailability of Contaminants in Soils and Sediments: Processes, Tools, and

Applications. Washington, National Research Council of the National Academies. (available at https:// www.nap.edu/catalog/10523/bioavailability-of-contaminants-in-soils-and- sediments-processes-tools-and).

Conesa, H., Faz, A., Arnaldos, R. (2007). Initial studies for the phytostabilization of a mine tailing from the Cartagena-La Union Mining District (SE Spain). *Chemosphere, 66,*38–44.

Cortés, A.M., Bridgwater, A.V. (2015). Kinetic study of the pyrolysis of miscanthus and its acid hydrolysis residue by thermogravimetric analysis. *Fuel Processing Technology, 138*, 184–193 Contents. http://dx.doi.org/10.1016/j.fuproc.2015.05.013.

Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E., Geissen, V. (2019). Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. *Science of The Total Environment, 671*, 411-420.

Costa, E.T.S., Guilherme, L.R.G., Melo, E.E.C., Ribeiro, B.T., Inácio, E.S.B., Severiano, E.C., Faquin, V., Hale, B.A. (2012). Assessing the tolerance of castor bean to Cd and Pb for phytoremediation purposes. *Biological Trace Elemement Research, 145*, 93–100.

Costanzo, S. D., Murby, J., Bates, J. (2005). Ecosystem response to antibiotics entering the aquatic environment. *Marine Pollution Bulletin, 51*, 218–223. https://doi.org/10.1016/j.marpolbul.2004.10.038.

Ćujić, M., Dragović, S., Đorđević, M., Dragović, R., Gajić, B. & Miljanić, Š. (2015). Radionuclides in the soil around the largest coal-fired power plant in Serbia: radiological hazard, relationship with soil characteristics and spatial distribution. *Environmental Science and Pollution Research, 22*(13), 10317–10330. https://doi.org/10.1007/s11356-014-3888-2.

Cunningham, S.D., Brti, W.R., Huang, J.W. (1995). Phytoremediation of contaminated soils. *Trends in Biotechnology, 13*, 393-397.

Cutright, T., Gunda, N., Kurt, F. (2010). Simultaneous hyperaccumulation of multiple heavy metals by *Helianthus annuus* grown in a contaminated sandy-loam soil. *International Journal of Phytoremediation, 12*, 562–73.

DalCorso, G., Manara, A., Furini, A. (2013). An overview of heavy metal challenge in plants: from roots to shoots. *Metallomics*, 5, 1117 - 1132.

Damartzis, T., Vamvuka, D., Sfakiotakis, S., Zabaniotou, A., (2011). Thermal degradation studies and kinetic modeling of cardoon (*Cynara cardunculus*) pyrolysis using thermogravimetric analysis (TGA). *Bioresource Technology, 102*, 6230-6238.

De Vivo, B., Lima, A., Siegel, F.R. (2004). *Geochimica Ambientale: metalli potenzialmente tossici*. Naples, Italy: Liguori editore.

Diacono, M., Montemurro, F. (2015). Effectiveness of organic wastes as fertilizers and amendments in salt-affected soils. *Agriculture, 5* (2), 221-230.

Díaz-Cruz, M.S., Barceló, D. (2005). LC–MS2 trace analysis of antimicrobials in water, sediment and soil. *Trends in Analytical Chemistry, 24* (7), 645–657. https://doi. org/10.1016/j.trac.2005.05.005

Dickinson, N.M., Baker, A.J.M., Doronila, A., Laidlaw, S., Reeves, R.D. (2009). Phytoremediation of inorganics: realism and synergies. *International Journal of Phytoremediation, 11*, 97-114.

Dincer, I., Zamfirescu, C. (2014). Chapter 3 - Fossil Fuels and Alternatives. *Advanced Power Generation Systems,3*, 95-141. https://doi.org/10.1016/B978-0-12-383860-5.00003-1

Domínguez, M. T., Montiel-Rozas, M. M., Madejón, P., Diaz, M. J., & Madejón, E. (2017). The potential of native species as bioenergy crops on trace-element contaminated Mediterranean lands. *Science of the Total Environment, 590–591*, 29–39. https://doi.org/10.1016/j.scitotenv.2017.03.018.

Dushenkov, V., Kumar, P.B.A.N., Motto, H., Raskin, I. (1995). Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. *Environmental Science & Technology, 29*, 1239– 1245.

Dushenkov, S., Mikheev, A., Prokhnevsky, A., Ruchko, M., Sorochinsky, B. (1999). Phytoremediation of radiocesium-contaminated soil in the vicinity of Chernobyl, Ukraine. *Environmental Science & Technology, 33*(3), 469-475.

EC. (1991). Council Directive 91/676/EEC of 12 December 19991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. [accessed 3 April 2020]. https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:31991L0676&from=EN.

El Kheir, S.B., Oubbih, J., Saidi, N., Bouabdli, A. (2008). Uptake and fixation of Zn, Pb, and Cd by *Thlaspi caerulescens*: application in the cases of old mines of Mibladen and Zaida (West of Morocco). *Arabian Journal of Geosciences, 1*, 87–95. https://doi.org/10.1007/s12517-008-0007-z.

E.P.A. (2000). Introduction to Phytoremediation, EPA Document 600-R-99-107, U.S. Environmental Protection Agency, National Risk Management Research Laboratory, Office of Research and Development, Cincinnati, Ohio 45268. Available in: http://www.epa.gov.

EPA. (2012). A Citizen ’ s Guide to Phytoremediation. A Citizen’S Guide To Drycleaner Cleanup, 1–6. [accessed 15 April 2020] https://drycleancoalition.org/download/citizens_guide_drycleaner_cleanup.pdf.

Ernst, W.H.O. (1996). Bioavailability of heavy metals and decontamination of soils by plants. *Applied Geochemistry, 11* (1/2), 163-167.

Ferreira, C.I., Calisto, V., Cuerda-Correa, E.M., Otero, M., Nadais, H., Esteves, V.I. (2016). Comparative valorisation of agricultural and industrial biowastes by combustion and pyrolysis. *Bioresources Technology, 218*, 918-925.

Freitas, E.V.S., Nascimento, C.W.A. (2009). The use of NTA for lead phytoextraction from soil from a battery recycling site. *Journal of Hazardous Materials, 171*, 833-837.

Frumin, G.T., Gildeeva, I.M. (2014). Eutrophication of water bodies- A global environmental problem. *Russian Journal of General Chemistry, 84* (13), 2483-2488. https://doi.org/10.1134/S1070363214130015

Geissen, V., Mol, H., Klumpp, E., Umlauf, G., Nadal, M., van der Ploeg, M., van de Zee, S.E.A.T.M. Ritsema, C.J. (2015). Emerging pollutants in the environment: A challenge for water resource management. *International Soil and Water Conservation Research, 3*(1), 57–65. https://doi.org/10.1016/j.iswcr.2015.03.002.

Gelleti, R., Jodice, R., Mauro, G., Migliardi, D., Picco, D., Pin, M., Simeoni, P. (2006). Energia dalle biomasse.Le Tecnologie, i Vantaggi per i Processi Produttivi, i Valori Economici e Ambientali (1st ed.), Vol. 24, pp. 187–214. Padriciano, Italy: Area Science Park.

Ghisari, M., Bonefeld-Jorgensen, E.C. (2009). Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. *Toxicology Letters, 189*(1), 67– 77. https://doi.org/10.1016/j.toxlet.2009.05.004.

Gisbert, C., Ros, R., De Haro, A., Walker, D.J., Bernal, M.P., Serrano, R., Navarro-Aviñó, J. (2003). A plant genetically modified that accumulates Pb is specially promising for phytoremediation. Biochemical and Biophysical Research Communications 303, 440-445.

Gomes, H.I. (2012). Phytoremediation for bioenergy: challenges and opportunities. *Environmental Technology Reviews,1*(1),59-66. https://doi.org.10.1080/09593330.2012.696715.

Gomez, L., Contreras, A., Bolonio, A., Quantana, J., Onate-Sanchez, L., Merino, I. (2019). Phytoremediation with trees. *Advances in Botanical Research, 89*(10), 281-321.

Good, A.G., Beatty, P.H. (2011). Fertilizing Nature: A Tragedy of Excess in the Commons. *PLoS Biology, 9*(8), 1-9. https://doi.org/10.1371/journal.pbio.1001124.

Gou Z.H., Miao X.F. (2010). Growth changes and tissues anatomical characteristics of giant reed (*Arundo donax* L.) in soil contaminated with arsenic, cadmium and lead. *Journal of Central South University of Technology,17*, 770−777.

Grčman, H., Vodnik, D., Velikonja-Bolta, Š., Leštan, D. (2003). Ethylenediaminedissuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. *Journal of Environmental Quality, 32*, 500-506.

Grčman, H., Lestan, D. (2003). Phytoextraction of lead, zinc and cadmium from soil by selected plants. *Plant Soil and Environment, 49*(12), 548-553.

Grippi, D., Clemente, R., Bernal, M.P. (2020). Chemical and bioenergetic characterization of biofuels from plant biomass: perspectives for Southern Europe. *Applied Sciences, 10*(10), 1-28. https:// doi.org/10.3390/app10103571.

Guzzella, L., Poma, G., De Paolis, A., Roscioli, C. Viviano, G. (2011). Organic persistent toxic substances in soils, waters and sediments along an altitudinal gradient at Mt. Sagarmatha, Himalayas, Nepal. *Environmental Pollution, 159*(10), 2552–2564. https:// doi.org/10.1016/j.envpol.2011.06.015

Hao, X., Chang, C., Travis, G.R., Zhang, F. (2003). Soil carbon and nitrogen response to 25 annual cattle manure applications. *Journal of Plant Nutrition and Soil Science, 166*(2), 239–245. https://doi.org/10.1002/jpln.200390035.

Hayes, D. G., Wadsworth, L. C., Sintim, H. Y., Flury, M., English, M., Schaeffer, S. and Saxton, A. M. (2017). Effect of diverse weathering conditions on the physicochemical properties of biodegradable plastic mulches. *Polymer Testing, 62*, 454-467.

Hernandez-Pinero, J.L., Maiti, R.K., Verde-Star, M.J., Garcia-Diaz, G., Nunez-Gonzalez, A., Cardenas-Avila, M.L., Foroughbakhch, R, (2002). Potential use of *Phaseolus vulgaris* L., *Avena sativa* L. and *Medicago sativa* L. in the removal of lead and cadmium from soil. *Crop Research Hisar, 24*(3), 449-452.

Hossain, M.F., White, S.K., Elahi, S.F., Sultana, N., Choudhury, M.H.K., Alam, Q.K., Rother, J.A. Gaunt, J.L. (2005).The efficiency of nitrogen fertiliser for rice in Bangladeshi farmers' fields, *Field Crops Research, 93*(I), 94-107. https://doi.org./10.1016/j.fcr.2004.09.017.

https://www.efsa.europa.eu/it/topics/topic/pesticides.

http://www.sapere.it/sapere/strumenti/studiafacile/scienza/Il-suolo/Il-suolo/La-composizione-dei-suoli.html).

Huang, J.W., Cunningham, S.D. (1996). Lead phytoextraction: species variation in lead uptake and traslocation. *New Phytologist, 134*, 75-84.

Huang, J.W., Chen, J., Berti, W.R., Cunningham S.D. (1997). Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environmental Science and Technology, 31*, 800-805-

Huang, C.L., Liao, W.C., Lai, Y.C. (2011). Cultivation studies of Taiwanese native *Miscanthus floridulus* lines. *Biomass Bioenergy, 35*, 1873-1877.

Hunce, S. Y., Clemente, R., Bernal, M.P. (2019). Energy production potential of phytoremediation plant biomass: *Helianthus annuus* and *Silybum marianum*. *Industrial Crops and Products, 135*, 206–216. https://doi.org/10.1016/j.indcrop.2019.04.029.

Husson, O. (2013). Redox potential (Eh) and pH as drivers of soil/plant/microorganism systems: a transdisciplinary overview pointing to integrative opportunities for agronomy. *Plant Soil, 362*(1-2), 389-417.

Iaboni, V., De Stefanis, P. (2003). La valorizzazione delle scorie di combustione dei rifiuti urbani. *Complessità Sviluppo, 1*, 146–159.

I.T.R.C. (1999). Phytoremediation Decision Tree, prepared by Interstate Technology & Regulatory Cooperation Working group, Phytoremediation Work Team. http://www.itrcweb.org

I.T.R.C. (2001). Phytotechnology Technical and Regulatory Guidance Document, prepared by Interstate Technology & Regulatory Cooperation Working group, Phytotechnologies Work Team. http://www.itrcweb.org.

I.T.R.C. (2009). Phytotechnology Technical and Regulatory Guidance and Decision Trees, Revised. PHYTO-3. Washington, D.C.: Interstate Technology and Regulatory Council, Phytotechnologies Team, Tech. Reg. Update. http://www.itrcweb.org

Jain, S., Sharma, M. (2012) Application of thermogravimetric analysis for thermal stability of *Jatropha curcas* biodiesel. *Fuel, 93*, 252–257.

Jeguirim, M., Dorge, S., Trouvé, G. (2010). Thermogravimetric analysis and characteristics of two energy crops in air atmosphere: *Arundo donax* and *Miscanthus giganteus*. *Bioresource Technology, 101*, 788–93.

Jones, A., Panagos, P., Barcelo, S., Bouraoui, F., Bosco, C., Dewitte, O., Gardi, C., Erhard, M., Hervás, J., Hiedere, R., Jeffery, S., Lükewille, A., Marmo, L., Montanarella, L., Olazábal, C., Petersen, J.E., Penizek, V., Strassburger, T., Tóth, G., Van Den Eeckhaut, M., Van Liedekerke, M., Verheijen, F., Viestova, E., Yigin, Y. (2012). *State of Soil in Europe,* pp. 1-78, Luxemburg: European Environment Agency. https://doi.org/10.2788/77361.

Kara, M., Yuzer, H., Sabah, E. and Celik (2003). Adsorption of cobalt from aqueous solutions onto sepiolite. *Water Research, 37,* 224–232.

Karaosmanoglu, F., Cift, B.D., Ergudenler, A.I. (2001). Determination of reaction kinetics of straw and stalk of rapeseed using thermogravimetric analysis. *Energy Sources, 23*,767-774.

Karmakar, R., Das, I., Dutta, D., Rakshit, A., 2016. Potential effects of climate change on soil properties: a review. *Science International, 4*(2), 51-73.

Kaur, R., Gera, P., Jha, M. K., & Bhaskar, T. (2018). Pyrolysis kinetics and thermodynamic parameters of castor (*Ricinus communis*) residue using thermogravimetric analysis. *Bioresource Technology*, *250,* 422–428. https://doi.org/10.1016/j.biortech.2017.11.077

Kidd, P., Mench, M., Álvarez-López, V., Bert, V., Dimitriou, I., Friesl-Hanl, W., Herzig, R., Janssen, J.O., Kolbas, A., Müller, I., Neu, S., Renella, G.O., Ruttens, A., Vangronsveld, J., Puschenreiter, M. (2015). Agronomic Practices for Improving Gentle Remediation of Trace Element- Contaminated Soils. *International Journal of Phytoremediation, 17*(11), 1005 - 1037.

Kidd, P., Barceló, J., Bernal, M.P., Navari-Izzo, F., Poschenrieder, C., Shilev, S., Clemente, R., Monterroso, C. (2009). Trace element behaviour at the root–soil interface: Implications in phytoremediation. *Environmental and Experimental Botany, 67,* 243-259.

Kocaoba, S. (2009). Adsorption of Cd(II), Cr(III) and Mn(II) on natural sepiolite. *Desalination*, *244*(1–3), 24–30. https://doi.org/10.1016/j.desal.2008.04.033.

Kolarikova, M., Havrland, B., & Ivanova, T. (2013). Energy Balance of Hemp (*Cannabis sativa* L.) Grown for Energy Purposes. *Agricultura Tropica et Subtropica, 46*(1), 10–15. https://doi.org/10.2478/ats-2013-0002.

Komprda, J., Komprdová, K., Sáňka, M., Možný, M., Nizzetto, L. (2013). Influence of Climate and Land Use Change on Spatially Resolved Volatilization of Persistent Organic Pollutants (POPs) from Background Soils. *Environmental Science and Technology*, *47*(13), 7052-7059. https://doi.org/10.1021/es3048784

Kos, B., Greman, H., Lestan, D. (2003). Phytoextraction of lead, zinc and cadmium from soil by selected plants. *Plant Soil Environmental, 49*, 548-553.

Kos, B., Lestan, D. (2003b). Influence of a biodegradable ([S,S]-EDDS) and non-degradable (EDTA) chelate and hydrogel modified soil water sorption capacity on Pb phytoextraction and leaching. *Plant and Soil*, *253,* 403-411.

Kramer, U. (2005). Phytoremediation: novel approaches to cleaning up polluted soils. *Current opinion in Biotechnology*, *16,* 133-141.

Kumar, P.B.A.N., Dushenkov, V., Motto, H., Raskin, I. (1995) Phytoextraction: The use of plants to remove heavy metals from soils. *Environmental Science and Technology, 29*(5), 1232 - 1238.

Kumar, G.P., Yadav, S.K., Thawale, P.R., Singh, S.K., Juwarkar, A.A. (2008). Growth of *Jatropha curcas* on heavy metal contaminated soil amended with industrial wastes and Azotobacter – a greenhouse study. *Bioresource Technology, 99,* 2078–2082.

Lehman, R., Cambardella, C., Stott, D., Acosta-Martinez, V., Manter, D., Buyer, J.S., Maul, J.E.,Smith, J.L., Collins, H.P., Halvorson, J.J., Kremer, R.J., Lunfgren, J.G., Ducey, T.F., Jin, V.L., Karlen, D.L. (2015). Understanding and enhancing soil biological health: the solution for reversing soil degradation. *Sustainability, 7,* 988-1027.

Licata, M., La Bella, S., Leto, C., Bonsangue, G., Gennaro, M. C., Tuttolomondo, T. (2017). Agronomic evaluation of Ethiopian mustard (*Brassica carinata* A. Braun) germplasm and physical-energy characterization of crop residues in a semi-arid area of Sicily (Italy).*Chemical Engineering Transactions,58,* 535–540. https://doi.org/10.3303/CET1758090

Lerda, D. (2011). *Polycyclic Aromatic Hydrocarbons (PAHs) Factsheet* (4 th ed.), pp. 34. Belgium: Joint Research Centre, European Commission, (available at https://ec.europa.eu/jrc/sites/jrcsh/files/Factsheet PAH_0.pdf).

Li, H., Q. Wang, Y. Cui, Y. Dong and P. Christie. (2005). Slow release chelate enhancement of lead phytoextraction by corn (*Zea mays* L.) from contaminated soil–a preliminary study. *Science of the Total Environment, 339,* 179-187.

Lima, A.C.R., Brussaard, L., Totola, M.R., Hoogmoed, W.B., de Goede, R.G.M. (2013). A functional evaluation of three indicator sets for assessing soil quality. *Applied Soil Ecology, 64,* 194–200.

Lin, C., Liu, J., Wang, R., Wang, Y., Huang, B. Pan, X. (2013). Polycyclic Aromatic Hydrocarbons in Surface Soils of Kunming, China: Concentrations, Distribution, Sources, and Potential Risk. *Soil and Sediment Contamination: An International Journal, 22*(7), 753–766. https:// doi.org/10.1080/15320383.2013.768201.

Linger, P., Müssig, J., Fischer, H., Kobert, J. (2002). Industrial hemp (*Cannabis sativa* L.) growing on heavy metal contaminated soil: fibre quality and phytoremediation potential. *Industrial Crops Production, 16,* 33–42.

Liu, D., Wu, X., Chang, Gu, B., Min, Y., Ge, Y., Shi, Y., Xue, H., Peng, C., Wu, J. (2012). Constructed wetlands as biofuel production systems. *Nature Climate Change, 2,* 190–4.

Luo, C., Shen, Z., Li, X. (2005). Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. *Chemosphere, 59,* 1-11

Madejón, P., Domínguez, M.T., Díaz, M.J., Madejón, E. (2016). Improving sustainability in the remediation of contaminated soils by the use of compost and energy valorization by *Paulownia fortunei*. *Science of the Total Environmental, 539,* 401-409.

Magistrelli, P., Bregante, M., De Robertis, S., Martella L., Paganetto, A., Qualich, P., Racah, J., Natali Sora, I., Gambale, F. (2002), Depurazione di suoli inquinati da metalli mediante fitoestrazione, Istituto di biofisica, CNR, Sezione di Genova, Parco Scientifico Tecnologico della Liguria, Progetto PhyLeS. Available at https://www.scribd.com/document/54790987/2-SUOLO-Metalli-Pesanti-e-Fitorimedio.

Martínez, M., Bernal, M.P., Almela, C., Vélez, D., García-Agustín, Serrano, R., Navarro-Aviñó, J. (2006). An engineered plant that accumulates higher levels of heavy metals than *Thlaspi caerulescens*, with yields of 100 times more biomass in mine soils. Chemosphere 64, 478-485.

Martínez-Alcalá, I., Clemente, R., Bernal, M.P. (2009). Metal availability and chemical properties in the rhizosphere of *Lupinus albus* L. growing in a high-metal calcareous soil. *Water Air and Soil Pollution, 201*, 283-293.

McGrath, S. P., Lombi, E., Gray, C. W., Caille, N., Dunham, S. J., Zhao, F. J. (2006). Field evaluation of Cd and Zn phytoextraction potential by the hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis halleri*. *Environmental Pollution, 141*(1), 115–125. https://doi.org/10.1016/j.envpol.2005.08.022

McIntyre, T. (2003). Phytoremediation of Heavy Metals from Soils. *Advances in Biochemical Engineering Biotechnology,78*, 105-108.

McLaughlin, M.J., Tiller, K.G., Naidu, R., Stevens, D.P. (1996) Review: The behaviour and environmental impact of contaminants in fertilizers. *Australian Journal of Soil Reserch, 34,* 1-54.

McKendry, P. (2002). Energy production from biomass (part 2): conversion technologies. *Bioresource Technology, 83,* 47-54.

Meers, E., Ruttens, A., Hopgood, M.J., Samson, D., Tack, F.M.G. (2005). Comparison of EDTA and EDDS as potential soil amendmens for enhanced phytoextraction of heavy metals. *Chemosphere, 58*, 1011-1022.

Mench, M., Lepp, N., Bert, V., Schwitzguébel, J.P., Gawronski, S. W., Schröder, P., Vangronsveld, J. (2010). Successes and limitations of phytotechnologies at field scale: outcomes, assessment and outlook from COST Action 859. *Journal of Soils and Sediments, 10*(6), 1039 - 1070.

Mendez, M.O., Maier, R.M. (2008). Phytoremediation of mine tailings in temperate and arid environments. *Environmental Science and Biotechnology, 7,* 47 - 59.

Mertens, J., Vervaeke, P., De Schrijver, A., Luyssaert, S. (2004). Metal uptake by young trees from dredged brackish sediment: limitations and possibilities for phytoextraction and phytostabilisation. *Science of the Total Environment, 326*, 209-215.

Miceli, A. (2001). Fitodecontaminazione e Risanamento dei suoli. *Ambiente Risorse Salute, 77,* 21-24.

Mirza, N., Mahmood, Q., Pervez, A., Ahmad, R., Farooq, M., Shah, M., Azim, M.R. (2010). Phytoremediation potential of *Arundo donax* in arsenic-contaminated synthetic wastewater. *Bioresource Technology, 101*(15), 5815-5819.

Mohapatra, D. P., Cledón, M., Brar, S. K., Surampalli, R. Y. (2016). Application of Wastewater and Biosolids in Soil: Occurrence and Fate of Emerging Contaminants. *Water, Air, & Soil Pollution 227*(3), 77.

Molas, J., Baran, S. (2004). Relationship between the chemical form of nickel applied to the soil and its uptake and toxicity to barley plants (*Hordeum vulgare* L.). *Geoderma, 122*(2/4), 247-255.

Moral, R., Pérez-Murcia, M.D., Pérez-Espinosa, A., Moreno-Caselles, J., Paredes, C., Rufete, B. (2008). Salinity, organic content, micronutrients and heavy metals in pig slurries from South-eastern Spain. *Waste Management, 28*, 367–371.

Morgan, R. (2013). Soil, heavy metals, and human health. In*: Soils and Human Health*, (Brevik, Burgess, ed.), pp. 59–82. Boca Raton, Fla: CRC Press.

Mosca, G., Vamerali, T., Ganis, A., Coletto, L., Bona, S. (2004). Miglioramento dell'efficienza agronomica della fitodecontaminazione di metalli pesanti. In: Fitoestrazione di Metalli Pesanti - Contenimento del Rischio Ambientale e Relazioni Suolo-Microrganismi-Pianta, (Forum ed.), Udine: Zerbi G, Marchiol L, 105-135.

Napper, I.E., Thompson, R.C. (2019). Environmental deterioration of biodegradable, oxo-biodegradable, compostable, and conventional plastic carrier bags in the sea, soil, and open-air over a 3-year period. *Environmental Science & Technology, 53*(9), 4775-4783.

Narasimha, M., Prasad, V., Pietrzykowski, M. (2020). *Climate Change and Soil Degradation*, pp. 51-88. Amsterdam: Elsevier.

Navari-Izzo, F., Quartacci, M.F., Sgherri, C. (2004). Fitoestrazione assistita e meccanismi di tolleranza ai metalli. In: Fitoestrazione di Metalli Pesanti - Contenimento del Rischio Ambientale e Relazioni Suolo-Microrganismi- Pianta. (Forum ed.), pp. 49-76. Udine, Italy: Zerbi, Marchiol.

Nehdi, I. A., Sbihi, H., Tan, C. P., Al-Resayes, S. I. (2013). Evaluation and characterisation of *Citrullus colocynthis* (L.) Schrad seed oil: Comparison with *Helianthus annuus* (sunflower) seed oil. *Food Chemistry,136*(2), 348–353. https://doi.org/10.1016/j.foodchem.2012.09.009.

Nsanganwimana, F., Pourrut, B., Mench, M., Douay, F. (2014). Suitability of Miscanthus species for managing inorganic and organic contaminated land and restoring ecosystem services. A review. *Journal of Environmental Management, 143*, 123-134. https://doi.org/10.1016/j.jenvman.2014.04.027.

Pan, B., Xing, B. (2012). Applications and implications of manufactured nanoparticles in soils: a review. *European Journal of Soil Science, 63*(4), 437–456. https://doi.org/10.1111/ j.1365-2389.2012.01475.x

Pandey, V. C., Bajpai, O., Singh, N. (2016). Energy crops in sustainable phytoremediation. *Renewable and Sustainable Energy Reviews, 54*, 58-73. https://doi.org/10.1016/j.rser.2015.09.078.

Papazoglou, E.G., Karantounias, G.A., Vemmos, S.N., Bouranis, D.L. (2005). Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to the heavy metals Cd and Ni. *Environment Internetional, 31*(2), 243–249. https://doi.org/10.1016/j.envint.2004.09.022.

Pardo, T., Bernal, M.P., Clemente, R. (2014). Efficiency of soil organic and inorganic amendments on the remediation of a contaminated mine soil: (I) Effects on trace elements and nutrients solubility and leaching risk. *Chemosphere, 107*, 121-128.

Pardo, T., Bernal, M.P., Clemente, R. (2017). The use of olive mill waste to promote phytoremediation. In: *Olive Mill Waste. Recent advances for Sustainable Management.* (C. M. Galanakis ed.), Vol. 9, pp. 183-204. Oxford: Academic Press.

Patent WO/2008/029423

Peralta-Videa, J.R., Gardea-Torresdey, J.L., Gomez, E., Tiemann, K.J., Parsons, J.G., Carrillo, G. (2002). Effect of mixed cadmium, copper, nickel and zinc at different pHs upon alfaalfa growth and heavy metal uptake. *Environmental Pollution, 119*(3), 291-301.

Petruzzelli, G., Pedron, F. (2007). Meccanismi di biodisponibilità nel suolo di contaminanti ambientali persistenti. In: *Impatto sulla Salute dei Siti Inquinati: Metodi e Strumenti per la Ricerca e le Valutazioni* (Comba, Bianchi, Iavarone, Pirastu ed.), pp. 68-75. Rome, Italy: Consiglio Nazionale della Ricerca.

Pidlisnyuk, V., Stefanovska, T., Lewis, E.E., Erickson, L.E., Davis, L.C. (2014). Miscanthus as a productive biofuel crop for phytoremediation. *Critical Review in Plant Sciences, 33*(1), 1–19.

Polettini, A., Pomi, R., Calcagnoli, G. (2009). Assisted Washing for Heavy Metal and Metalloid Removal from Contaminated Dredged Materials. *Water, Air & Soil Pollution, 196,* 183–198. https://doi.org/10.1007/s11270-008-9767-z.

Pollard, A.J., Reeves, R. D., Baker, A.J.M. (2014). Facultative hyperaccumulation of heavy metals and metalloids. *Plant Science, 8*(17), 217 - 218.

Pretty, J.N., Mason, C.F., Nedwell, D.B., Hine, R.E., Leaf, S., Dils, R. (2003). Enviromental Costs of Freshwater Eutrophication in England and Wales. *Environmental Science & Technology, 37*(2), 201-208. https://doi.org/10.1021/eso20793k

Pulford, I.D., Watson, C. (2003). Phytoremediation of heavy metal contaminated by trees - a review. *Environment International, 29*(4), 529-540.

Puschenreiter, M., Horak, O., Friesl, W., Hartl, W. (2005). Low-cost agricultural measures to reduce heavy metal transfer into the food chain - a review. *Plant, Soil and Environment, 51*(1), 1–11.

Quintella, C. M., Mata, A. M. T., Lima, L. C. P. (2019). Overview of bioremediation with technology assessment and emphasis on fungal bioremediation of oil contaminated soils. *Journal of Environmental Management, 241*, 156-166. https://doi.org/10.1016/j.jenvman.2019.04.019.

Rahman, M.A., Hasegawa, H., Kadohashi, K., Maki, T., Ueda, K. (2009). Hydroxyiminodisuccinic acid (HIDS): A novel biodegradable chelating ligand for the increase of iron bioavailability and arsenic phytoextraction. *Chemosphere, 77,* 207-213.

Rakhmetov, DB. (2007) Role of mnew cultures in bioenergy. *Science Release Nature Agriculture University*, *116*, 13–20 (in Ukrainian).

Raskin, I., Ensley, R.D., Burt, D. (1999). Phytoremediation of Toxic Metals: Using Plants to Clean the Environment. *Journal of Plant Biotechnology, 1*(1), 304. https://doi.org/10.1016/S0958-1669(97)80106-1.

Rillig, M. C., Ingraffia, R., de Souza, A., Machado, A. (2017). Microplastic Incorporation into Soil in Agroecosystems. *Frontiers in Plant Science, 8*, 1805.

Rizzi, L., Petruzzelli, G., Poggio, G., Guidi, G.V. (2004). Soil physical changes and plant availability of Zn and Pb in a treatability test of phytoextraction. *Chemosphere, 57*(9), 1039-1046.

Rodriguez-Eugenio, N., McLaughlin, M., Pennock, D. (2018). *Soil Pollution: a hidden reality*, pp. 142. Rome, Italy: FAO.

Romkens, P., Bouwman, L., Japenga, J., Draaisma, C. (2002). Potentials and drawbacks of chelate-enhanced phytoremediation of soils. *Environmental Pollution,116,*109–121.https://doi.org/ 10.1016/s0269-7491(01)00150-6. PMID: 11808546.

Ružbarský, J., Müller, M., Marecek, J., & Geršl, M. (2014). *Jatropha curcas*-analysis of gross calorific value. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,62*(6), 1381–1384. https://doi.org/10.11118/actaun201462061381

Salt, D.E., Smith, R.D., Raskin, I. (1998). Phytoremediation. *Annual Review of Plant Physiology, 49*, 643-668. https://doi.org/10.1146/annurev.arplant.49.1.643.

Santos, F.S., Hernández-Allica, J., Becerril, J.M., Amaral- Sobrinho, N., Mazur, N., Garbisu, G. (2006). Chelate assisted phytoextraction of metal polluted soils with Brachiaria decumbens. *Chemosphere, 65,* 43-50.

Sarret, G., Vangronsveld, J., Manceau, A., Musso, M., D'Haun, J., Menthonnex, J.J., Hazemann, J.L. (2001). Accumulation forms of Zn and Pb in *Phaseolus vulgaris* in the presence and absence of EDTA. *Environmental Science and Technology, 35,* 2854-2859.

Sas-Nowosielska, A., Kucharski, R., Małkowski, E., Pogrzeba, M., Kuperberg, J. M., Kryński, K. (2004). Phytoextraction crop disposal - An unsolved problem. *Environmental Pollution,128*(3), 373–379. https://doi.org/10.1016/j.envpol.2003.09.012

Schmidt, U. (2003). Enhancing phytoextraction: The effect of chemical soil manipulation on mobility, plant accumulation and leaching of heavy metals. *Journal of Environmental Quality, 32,* 1939-1954.

Schnoor, J.L. 1998. *Phytoremediation* (Schnoor, Ph. D., P.E., DEE, ed.), pp. 6. Pittsburgh: Ground-Water Remediation Technology Analysis Center.

Schulz, R., Bundschuh, M., Gergs, R., Bruehl, C.A., Diehl, D., Entling, M.H., Fahse, L., Frör, O., Jungkust, H.F., Lorke, A., Schäfer, R.B., Schaumann, G.E., Schwenk, K. (2015). Review on environmental alterations propagating from aquatic to terrestrial ecosystems. *Science of the Total Environment, 538,* 246-261.

Shen, Z.G., Li, X.D., Wang, C.C., Chen, H.M., Chua, H. (2002). Lead Phytoextraction from contaminated soil with high-biomass plant species. *Journal of Environmental Quality, 31,* 1893-1900.

Sintim, H. Y., Flury, M. (2017). Is Biodegradable Plastic Mulch the Solution to Agriculture's Plastic Problem?. *Environmental Science & Technology, 51*(3), 1068-1069.

Smith, S.L., Thelen, K.D., MacDonald, SJ. (2013). Yield and quality analyses of bioenergy crops grown on a regulatory brownfield. *Biomass Bioenergy,49,*123–30.

Soleimani, M., Akbar, S., Hajabbasi, M.A. (2011). *Enhancing phytoremediation efficiency in response to environmental pollution stress*. In: Plants and Environment (Vasanthaiah ed.), pp- 1-16. Shangai, China: Intech. https://doi.org/10.5772/22267.

St Clair, S., Hillier, J., Smith, P. (2008) Calculating the pre-harvest greenhouse gas costs of energy crops.*Biomass and Bioenergy, 32*, 442-452.

Steffan, J.J., Brevik, E.C., Burgess, L., Cerdà, A. (2018). The effect of soil on human health: An overview. *European Journal of Soil Science, 69*, 159-171. https://doi.org/ 10.1111/ejss.12451

Stigliani, W.M., Doelman, P., Salomons, W., Schulin, R., Smidt, G.R. (1991). Chemical time bombs: predicting the unpredictable. *Environment: Science and Policy for Sustainable Development, 33*(4), 4-30.

Suthan Suthersan S. (2002). Natural and Enhanced Remediation Systems (1st ed.), pp. 440. Boca Raton, Florida: Lewis Publishers.

Tamura, H., Honda, M., Sato, T., Kamachi, H. (2005). Pb hyperaccumulation and tolerance in common buckwheat (*Fagopyrum esculentum* Moench). *Journal of Plant Research, 118*, 355-359.

Tandy, S., Schulinn R., Nowack, B. (2006). The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflower. *Chemosphere*, *62*, 1454-1463.

Tangahu, B.V., Abdullah, S.R.S., Basri, H., Idris, M., Anuar, N., Mukhlisin, M. (2011). A Review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering*, *2011*, 31. https://doi.org/10.1155/2011/939161.

Técher, D., Laval-Gilly, P., Henry, S., Bennasroune, A., Formanek, P., Martinez-Chois, C., Falla, J. (2011). Contribution of *Miscanthus x giganteus* root exudates to the biostimulation of PAH degradation: An in vitro study. *Science of the Total Environment, 409*(20), 4489–4495. https://doi.org/10.1016/j.scitotenv.2011.06.049.

Thakur, L. S., Varma, A. K., Mondal, P. (2018). Analysis of thermal behavior and pyrolytic characteristics of vetiver grass after phytoremediation through thermogravimetric analysis. *Journal of Thermal Analysis and Calorimetry*, *131*(3), 3053–3064. https://doi.org/10.1007/s10973-017-6788-0.

Tóth, G., Hermann, T., Da Silva, M.R., Montanarella, L. (2016). Heavy metals in agricultural soils of the European Union with implications for food safety. *Environment International, 88*, 299-309.

Trakal, L., Martínez-Fernández, D., Vítková, M., Komárek, M. (2015). Phytoextraction of Metals: Modeling Root Metal Uptake and Associated Processes. In: *Phytoremediation*. Ansari A., Gill S., Gill R., Lanza G., Newman L. (eds), pp. 69-83. Switzerland: Springer International Publishing.

Truong, P.N. Baker, D. (1998). Vetiver grass system for environmental protection. Technical Bulletin No. 1998/1. Australia: Pacific Rim Vetiver Network. Office of the Royal Development Projects Board.

Tsao, D.T. (2003). Overview of Phytotechnologies, In: *Phytoremediation, Advances in biochemical engineering biotechnology*, 78, 1-50. Berlin: Springer. https://doi.org/10.1007/3-540-45991-X_1

USEPA. (2012). Phthalates Action Plan. Retrieved from https://www.epa.gov/sites/ production/files/2015-09/documents/phthalates_actionplan_revised_2012-03-14. pdf.

USEPA, (2014). Persistent Organic Pollutants: A Global Issue, A Global Response. Retrieved from https://www.epa.gov/international- cooperation/persistent-organic-pollutants-global-issue-global-response.

Vamerali, T., Bandiera, M., Coletto, L., Zanetti, F., Dickinson, N.M., Mosca, G. (2009) Phytoremediation trials on metal and arsenic contaminated pyrite wastes (Torviscosa, Italy). *Environmental Pollution, 157*, 887 - 894.

Vandenhove, H., Eyckmans, T., Van Hees, M. (2005). Can barium and strontium be used as tracers for radium in soil - Plant transfer studies?. *Journal of Environmental Radioactivity, 81*, 255–267. https://doi.org/10.1016/j.jenvrad.2004.01.039.

Van der Ent, A., Baker, A.J.M., Reeves, R.D., Pollard, A.J., Schat H. (2013). Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. *Plant and Soil, 362*, 319 - 334.

Van Ginneken, L., Meers, E., Guisson, R., Ruttens, A., Elst, K., Tack, F.M.G., Vangronsveld, J., Diels, L., Dejonghe, W. (2007). Phytoremediation for heavy metal-contaminated soils combined with bioenergy production. *Journal of Environmental Engineering and Landscape Management, 15* (4), 227-236.

Van Oosten, M.J, Maggio, A. (2015). Functional biology of halophytes in the phytoremediation of heavy metal contaminated soils. *Environmental and Experimental Botany, 111*, 135 - 146.

Várhegyi, G., Chen, H., Godoy, S. (2009). Thermal decomposition of wheat, oat, barley, and *Brassica carinata* straws. A kinetic study. *Energy and Fuels, 23*(2), 646–652. https://doi.org/10.1021/ef800868k.

Vitousek, P.M., Naylor, R., Crews, T., Davis, M.B., Drinkwater, L.E., Holland, E., Johnes, P.J., Katzenberg, J., Martinelli, L.A., Matson, P.A., Nziguheba, G., Ojima, D., Palm, C.A., Robertson, G.P., Sanchez, P.A., Townsend, A.R., Zhang, F.S. (2009). *Nutrient Imbalances in Agricultural Development. Science, 324* (5934), 1519-1520. https://doi.org/10.1126/science.1170261.

Wallova, G., Kandler, N. Wallner, G. (2012). Monitoring of radionuclides in soil and bone samples from Austria. *Journal of Environmental Radioactivity, 107*, 44–50. https://doi.org/10.1016/j.jenvrad.2011.12.007

Wang, L., Sun, X., Li, S., Zhang, T., Zhang, W., Zhai, P. (2014). Application of organic amendments to a coastal saline soil in north China: effects on soil physical and chemical properties and tree growth. *PLOS ONE, 9* (2), 89-185.

Weithmann, N., Möller, J. N., Löder, M. G. J., Piehl, S., Laforsch, C., Freitag, R. (2018). Organic fertilizer as a vehicle for the entry of microplastic into the environment. *Science Advance, 4*(4), 1-8. https://doi.org/ 10.1126/sciadv.aap8060.

Wenger, K., Kaiser, A., Gupta, S.K., Furre, G., Schulin, R. (2002). The comparison of NTA and elemental sulfur as potential soil amendments. *Soil and Sediment Contamination, 1*, 655-672.

Wilson, M., Bell, N. (1996). Acid deposition and heavy metal mobilization. *Applied Geochemistry, 11* (1-2), 133-137.

Withers, P.J.A., Sylvester-Bradley, R., Jones, D.L., Haley, J.R., Talboys, P.J. (2014). Feed the Crop Not the Soil: Rethinking Phosphorus Management in the Food Chain.

Environmental Science & Technology, 48(12), 6523-6530. https://doi.org/10.1021/es501670j.

Wong, M.H. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere, 50*, 775-780.

Yadav, S. K., Juwarkar, A. A., Kumar, G. P., Thawale, P. R., Singh, S. K., Chakrabarti, T. (2009). Bioaccumulation and phyto-translocation of arsenic, chromium and zinc by *Jatropha curcas* L.: Impact of dairy sludge and biofertilizer. *Bioresource Technology,100*(20), 4616–4622. https://doi.org/10.1016/j.biortech.2009.04.062.

Yang, M., Xiao, X. Y., Miao, X. F., Guo, Z. H., Wang, F. Y. (2012). Effect of amendments on growth and metal uptake of giant reed (*Arundo donax* L.) grown on soil contaminated by arsenic, cadmium and lead. *Transactions of Nonferrous Metals Society of China (English Edition), 22*(6), 1462–1469. https://doi.org/10.1016/S1003-6326(11)61342-3.

Yaron, B., Dror, I. Berkowitz, B. (2012). *Soil-Subsurface Change*, pp. 39-74. Berlin Heidelberg: Springer. (available at https://link.springer.com/10.1007/978-3-642-24387-5*)*

Yen, T.H., Lin-Tan, D.T. Lin, J.L. (2011). Food safety involving ingestion of foods and beverages prepared with phthalate-plasticizer-containing clouding agents. *Journal of the Formosan Medical Association, 110*(11), 671–684. https://doi.org/10.1016/j.jfma.2011.09.002.

Zabaniotou, A., Ioannidou, O., Antonakou, E., Lappas, A. (2008). Experimental study of pyrolysis for potential energy, hydrogen and carbon material production from lignocellulosic biomass. International *Journal of Hydrogen Energy, 33*, 2433–2444. http://dx.doi.org/10. 1016/j.ijhydene.2008.02.080.

Zerbi, G., Marchiol, L. (2004). Fitoestrazione di Metalli Pesanti - Contenimento del Rischio Ambientale e Relazioni Suolo- Microrganismi-Pianta. Zerbi G, Marchiol L(Eds.), pp. 9-36. Udine, Italy: Forum Editrice.

Zhang, C., Yu, Z.G., Zeng, G.M., Jiang, M., Yang, Z.Z., Cui, F. (2014). Effects of sediment geochemical properties on heavy metal bioavailability. *Environment International, 73*, 270-281.

Zhao, F., Yang, W., Zeng, Z., Li, H., Yang, X., He, Z., Gu, B., Rafiq, M.T., Peng, H. (2012). Nutrient removal efficiency and biomass production of different bioenergy plants in hypereutrophic water. *Biomass Bioenergy, 4*, 212–8.

Zhu, J.H., Li, X.L., Christie, P., Li, J.L. (2005). Enviromental implication of low nitrogen use efficiency in excessively fertilized hot pepper (*Capsicum frutescens* L.) cropping systems. *Agriculture, Ecosystems & Environment, 3* (1-4), 70-80. https://doi.org./10.1016/j.agee.2005.04.025.

CHAPTER 3. POTENTIAL FOR BIOENERGY PRODUCTION OF PLANT BIOMASS FROM TRACE-ELEMENTS CONTAMINATED SOILS UNDER MEDITERRANEAN CLIMATE CONDITIONS

CHAPTER 3

POTENTIAL FOR BIOENERGY PRODUCTION OF PLANT BIOMASS FROM TRACE-ELEMENTS CONTAMINATED SOILS UNDER MEDITERRANEAN CLIMATE CONDITIONS

1. INTRODUCTION

The mining district of Cartagena-La Unión is a mountain formation that extends from east to west along 26 km of coast from the city of Cartagena to the end of Palos Cope, passing through the municipality of La Unión, in the Region of Murcia (Spain). In ancient times, this area was exploited intensely for ores containing silver, lead, zinc and other metallic minerals. After the Spanish Civil War, a large open-pit mining operation caused serious environmental problems up until the definitive cessation of the mining activities in 1990. As a consequence of this vast historical process of industrial exploitation and intense human activity, the landscape of the Cartagena-La Unión area has been marked and transformed into a sterile area of around 50 km^2, the old vegetation cover has disappeared along with habitat and numerous species (Conesa and Schulin, 2010). In addition, the Portmán bay resulted completely collapsed, as it received about 33 million cubic meters of toxic mining wastes, the seashore advanced up to 700 meters and the seabed was affected by sediments loaded with potentially toxic trace elements (TEs) up to 12 km offshore.

The tailings of the mining district of Cartagena - La Unión can be classified in (Conesa et al., 2006; Conesa et al., 2008a):

- Acid tailings, with a pH of about 3, characterized by the presence of oxidized and reduced minerals (such as magnetite and sphalerite), which have shown high soluble concentrations of metals such as Zn or Cd (up to 14 % of total Zn has been detected in water extracts);

- Tailings with neutral pH, characterized by the presence of non-oxidizing minerals such as quartz and gypsum, with low solubility of metals but easily extractable with chelating agents such as EDTA.

These tailings contain mainly Fe (11-26 %), Si (8-13 %), Al (2-4 %) and S (2-3 %), and other TEs such as Zn (9100 mg kg^{-1}), As (1900 mg kg^{-1}), Cd (10–34 mg kg^{-1}) and Pb (5000–7000 mg kg^{-1}). However, low values (between 77-160 mg kg^{-1} Cu, 28-150 mg kg^{-1} Pb and 93-400 mg kg^{-1} Zn) have been reported for local cultivated land (Conesa, 2003).

Mining is one of the most relevant industrial activities that generate a significant environmental impact, even many years after the closure of the mining activities themselves (Fanfani et al., 2000; Conesa and Schulin, 2010).The main environmental problems that mining activities provoke are due to:

- *Erosion of the soils*. The excavation of open cast mines makes the soil rocky and therefore more hostile to the growth of vegetation. In particular, in the mining district of Cartagena-La Unión about 70 % of the area and about 80 % of the total volume of waste deposits are made up of mine spoil and mine tailings. Mine tailings are generally characterized by low pH values, high concentrations of heavy metals and metalloids, lack of nutrients, low water retention capacity and high electrical conductivity. These factors make the environment adverse for plant growth and, consequently, the surface soils remain generally bare of vegetation and exposed to erosion (Conesa and Schulin, 2010).

- *Release of potentially toxic TEs*. Another major problem related to erosion, due mostly to the action of wind and rain water, is the release of TEs from mining sites over time, which can lead to widespread contamination over large areas.

- *Air pollution*. Exposure to tailings emitted dust can pose health risks to nearby populations.

- *Chemical pollution of surface and underground water and contamination of the food chain.* The use of local groundwater for the irrigation of private gardens and agricultural land can generate an unwanted transfer of potentially toxic TEs to crops and, therefore, enter the food cycle (Conesa et al., 2010). Despite the soils showed elevated metal concentrations (510 mg kg^{-1} Pb and 910 mg kg^{-1} Zn), the concentrations found in plant leaves were rather low(Pb < 20 mg kg^{-1}; Cu 10-28 mg kg^{-1}; Zn 60-85 mg kg^{-1}). The high pH of local soils seemed to limit the accumulation of metals by the plants (Navarro et al., 2006).

- *Reduction of biodiversity*. For instance, the dispersion of mining waste into the surrounding environment and the consequent absorption of pollutants by local ecosystems in Portmán Bay has led to colonization by spontaneous plant species that show adaptation to metal contamination and saline soils (Conesa and Faz, 2009). Halophytic species like *Sarcocornia fruticosa*, *Salicornia ramossisima* and *Arthrocnemum macrostachyum*, or other species typical of saline areas like *Phagmites australis* and *Tamarix canariensis*, can be often found in those soils (Conesa and Faz, 2009). Furthermore, mining waste discharged into the Mar Menor lagoon and the Mediterranean Sea are known to strongly influence their respective biological and faunal communities (María-Cervantes et al., 2009; Sanchiz et al., 2000; Deheyn et al., 2000).

In the last twenty years, the growing awareness of the need to combine production activities and economic development with the protection of the environment has stimulated numerous studies and initiatives to understand the mechanisms of dispersion of toxic elements from mining activities, especially in countries such as the USA, Canada and Australia. Such activities have tried to foresee and prevent the consequences, and/or propose suitable containment and remediation measures. In this regard, plant technologies have proven to be an effective and promising low-cost tool for the in *situ* stabilization of metal contaminated sites. The Cartagena-La Unión mining district, as most former mining areas, is considered suitable for non-invasive remediation technologies, such as phytostabilization (Mendez and Maier, 2008), due to the huge areas to be treated (Conesa et al., 2008b). Through phytostabilization, the establishment of a self-sufficient vegetative cover reduces the risk of an uncontrolled transfer of pollutants into the environment.

In order to address the increasing demand for energy production and at the same time deal with environmental protection issues, the European Union has set the goal of increasing the share of renewable energy to 20 %, among other objectives of the “Europe 2020 Strategy”. Bioenergy is the main renewable energy source in the world. In total, firewood represents more than 56 % of the total bioenergy; vegetable waste is the second largest source of bioenergy in Asia (122 Mtoe) and Africa (76 Mtoe), biogas in Europe (15.8 Mtoe), biogasoline in North and Central America (28.6 Mtoe), and bagasse (34.9 Mtoe) and biogasoline (15.7 Mtoe) in South America.

The studies carried out to date show that some plant species have particular physical and chemical characteristics for their use as biomass crops for bioenergy production (Grippi et al., 2020), with clear advantages both in terms of energy production and at the economic level. The use of plant biomass for the production of (bio)energy can therefore be considered a suitable and environmentally friendly option to achieve the desired renewable energy share. Bibliographic research has shown that plants belonging to the families of Poaceae, Asteraceae, Brassicaceae and Graminaceae, with particular consideration for *Sorghum*, *Miscanthus* and *Arundo donax*, all belonging to the family of Poaceae, have characteristics suitable for their use as biofuels (Grippi et al., 2020; see Chapter 1, Section 5). In addition, the use of contaminated soils to produce energy crops may help to avoid competition with food production (Rowe et al., 2009), and the use of native species as bioenergy crops would limit the introduction of non-native species into the soil ecosystem to be remediated. This is particularly relevant for phytostabilization, as the plants have low TE concentration in their harvestable tissues. Species such as milk thistle (*Silybum marianum*), *Dittrichia viscosa*, *Piptatherum miliaceum* and *Nicotiana glauca* have been tested in soils with high TE content and for the production of bioenergy (Bernal et al., 2019; Domínguez et al., 2017a,b; Hunce et al., 2019).

Therefore, the objectives of this study were:

- To identify native plant species that have characteristics suitable for the production of bioenergy and that, at the same time, can tolerate high levels of potentially toxic TEs (heavy metals and metalloids) to be used in the recovery of contaminated soils through promising recovery technologies (phytostabilization);

- To define the potential for thermal and biogas production of plant biomass from TEs contaminated mine soils in order to determine the usefulness of these species for bioenergy production.

2. MATERIALS AND METHODS

2.1. Plant sampling and sample preparation

Different plant species growing spontaneously in the areas of El Gorguel and El Llano del Beal, within the Sierra Minera of La Unión-Cartagena, were sampled by cutting the aerial part (shoots) of several individuals of fully developed plants. The species included *Arundo donax* L. (Poaceae), *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae), *Piptatherum miliaceum* (L.) Coss. (Poaceae), *Dittrichia viscosa* (L.) Greuter (Asteraceae), *Zygophyllum fabago* L. (Zygophyllaceae), *Bituminaria bituminosa* L. Stirton (Fabaceae), *Atriplex halimus* L. (Chenopodiaceae) and *Foeniculum vulgare* Mill. (Apiaceae). The fresh samples were taken to the laboratory, washed with deionized water, dried at 60 ºC during 48 h and ground to < 0.5 mm in a stainless steel laboratory mill (A10 IKA Labortechnik, Staufen, Germany) prior to experiments and analysis.

2.2. Chemical analyses

The chemical composition of plant samples was determined for macro- and micronutrients and TEs by inductively coupled plasma optical emission spectroscopy (ICP-OES; THERMO ICAP 6500 DUO instrument, Thermo Scientific), after microwave-assisted digestion (ETHOS1, Milestone) with H_2O_2 and HNO_3 (1:4 v/v). The analytical accuracy was verified with a certified reference material (NCS DC

73349). Total solids (TS) were determined by drying at 105 °C, and volatile solids (VS) by ashing at 550 ºC in a muffle furnace (Carbolite AAF 11/3, Hope Valley, England) according to EPA method 1684 (EPA, 2001). The concentration of lignin and holocellulose were determined according to the American National Standard method (ANSI and ASTM, 1977a,b). The elemental analysis of C, N, S and H was carried out using a LECO CHNS-932 analyzer. The concentration of soluble carbohydrates was determined through the anthrone method (Hedge and Hofreiter, 1962). The higher heating value (HHV; MJ kg^{-1}) of the plant biomass was calculated with the data obtained from the elemental analysis, using the method of Sheng and Azevedo (2005), according to the following equation:

$$\text{HHV (MJ kg}^{-1}) = -1.3675 + 0.3137 \times \%C + 0.7009 \times \%H + 0.0318 \times \%O \qquad \text{(Eq. 1)}$$

All the analyses were done at least in duplicate.

2.3. Anaerobic degradation

Biogas production of the plant samples was determined through anaerobic digestion, using the ANKOM Gas Production System with 305 and 310 mL bottles (ANKOM^{RF}, ANKOM Technology, Macedon, NY, USA). With this system, the increase in pressure caused by the generation of gas in the absence of oxygen is automatically recorded, through which the volume of biogas produced is then calculated and expressed as the volume in mL of CH_4 per gram of VS. The anaerobic inoculum was collected from an urban wastewater plant under mesophilic conditions (reactor capacity 7612 m^3; hydraulic retention time 29.7 days) and previously incubated at 37 °C for 24 hours. The composition of the inoculum was: pH 7.37 ± 0.03, electrical conductivity 14.78 ± 3.58 dS m^{-1}, total solids 21.67 ± 4.13 g L^{-1} and volatile solids 14.02 ± 2.12 g L^{-1}.

Then, 0.5 g of each plant sample was mixed with 100 mL of pre-incubated inoculum in individual bottles (VS ratio 1:3, substrate:inoculum). The mixtures were then incubated in the absence of oxygen at a temperature of 37 °C under continuous stirring. A control sample without plant material and a positive control containing 0.5 g of cellulose were also used. The samples were run in duplicate. Before starting the system, the anaerobic conditions were obtained by filling the headspace of the containers with an N_2/CO_2 mixture (80:20, v:v). The pressure generated inside the containers was recorded at 15 min. intervals throughout the experiment. The results were expressed as volume of biogas per unit of volatile solids of the plant. The volume of biogas produced was calculated using Avogadro's ideal gas law, as shown below:

$$\text{Biogas (mL g}^{-1}\text{)} = [V_H \times (P_s - P_i) \times R \times T \times 22.414 \times 1000] / m \qquad \text{(Eq. 2)}$$

Where V_H is the volume of the headspace (L); P_s is the pressure in kPa of the sample; P_i is the pressure in kPa of the inoculum; R is the gas constant (8.314463 L Pa K^{-1} mol^{-1}); T is the temperature in Kelvin (ºK); m is the weight of the sample (g of VS).

The experiment finished when the biogas production reached almost the maximum in most samples (14-16 days). Then, biogas aliquots (in duplicate) from each sample were taken from each bottle through the septa port using a 10 mL glass syringe, and samples were kept in vacuum containers prior to analysis (Hansen et al., 2004). These gas samples were considered representative of biogas composition as the generated gases accumulated in the bottles throughout the incubation period. The percentage of methane (CH_4) in the biogas samples was analyzed using a Gas Chromatography system (Agilent 490 Micro GC, Santa Clara, CA, USA). The biochemical methane potential (BMP) of each plant material was calculated from the biogas production potential and the percentage of CH_4. The theoretical BMP (TBMP) was calculated from the plant characteristics using a stoichiometric equation based on the concentrations of lignin, holocellulose, soluble carbohydrates, proteins (calculated by multiplying N concentration by 6.25) and fats (Triolo et al., 2012):

$$\text{TBMP} = ([\text{lipid}] \times 1014 + [\text{protein}] \times 496 + [\text{carbohydrate}] \times 415 + [\text{lignin}] \times 727) \times 0.001 \text{ (Eq. 3).}$$

The lipid concentration of the aerial vegetative parts was estimated as 3.09 g kg^{-1} dry matter (Triolo et al., 2012; Hunce et al., 2019) for the TBMP calculation (Eq. 3). The ratio BMP/TBMP was used as the anaerobic biodegradability.

The experimental results of the biogas production were described by a first order kinetic model:

$B_m = B_0 \times (1\text{-}e^{-Kd\times t})$ (Eq. 4)

Where B_m is the biogas (mL g^{-1}VS) produced at time t; B_0 indicates the maximum biogas production potential (mL g^{-1}VS); and K_d is the anaerobic degradation rate constant. The experimental results were fitted to the model by the nonlinear least square technique (Marquardt-Levenberg algorithm), using the software Sigma Plot v.14.0 (Systat Software Inc.).

2.4. Statistical analysis

The effect of plant species on the plant composition, HHV and on the results of anaerobic degradation was determined by a one-way ANOVA. Differences between means were determined using Tukey’s test at $P<0.05$. Before the statistical analysis, the data were tested for normality using the Kolmogorov–Smirnov test. Pearson’s correlation coefficients between the degradation parameters and the TE concentrations in the plants were also determined (IBM SPSS Statistics 25 software).

3. RESULTS AND DISCUSSION

3.1. Plant composition

Nitrogen (N), phosphorus (P), potassium (K), and sulfur (S) are the main macronutrients, these elements are essential to plants and they are needed in large quantities for crop growth (Sathya et al., 2016). They are naturally present in the environment; however, their availability to plants in soils can be limited due to their natural forms. Therefore, fertilizers are applied to increase the crop yields but, an excessive fertilization and macronutrients intake can lead to a deficiency of micronutrients such as copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn), which act as a cofactor for several enzymes involved in different metabolic processes (Barker and Pilbeam, 2015).

The highest K concentration occurred in *A. halimus* and the lowest in *P. australis*, with significant differences between the plant species (Table 3.1). The concentrations of P in the plants were within a narrow range, with the highest value in *Z. fabago* and the lowest also in *P. australis*, which may result from low nutrient transfer from roots to shoots of the latter plant. The nutrient concentrations in the aboveground tissues of *P. australis* usually decrease during the growing season as a result of the “dilution effect”, and also due to their slow transfer from the below ground tissues at the end of the growing season (Zhao et al., 2013). The concentrations of K and P in *D. viscosa* were slightly higher than the values found by Martinez-Fernandez et al. (2014) (K 6.2 g kg^{-1}; P 0.4 g kg^{-1}), while the Ca, Mg and Na concentrations were much lower in the present experiment (Ca 23.4 g kg^{-1}; Mg 11.6 g kg^{-1}; Na 22.0 g kg^{-1}; Martinez-Fernandez et al., 2014). The concentrations of Ca and Mg in *B. bituminosa* (Ca 19.6 g kg^{-1}; Mg 8.2 g kg^{-1}) and in *P. miliaceum* (Ca 4.9 g kg^{-1}; Mg 7.0 g kg^{-1}) were all clearly lower than those reported by the same authors. The N concentrations were the highest in *Z. fabago* and *B. bituminosa* (Table 3.6), the later with the lowest C/N ratio. However, *D. viscosa* and *P. australis* showed the lowest N values, which were significantly different from the rest of the samples.

Table 3.1. Macronutrient and sodium concentrations in the plants used in the experiment (average ± se; n = 2). For nitrogen see Table 3.6.

Plants	**K ($g\ kg^{-1}$)**	**P ($g\ kg^{-1}$)**	**Ca ($g\ kg^{-1}$)**	**Mg ($g\ kg^{-1}$)**	**Na ($g\ kg^{-1}$)**
D. viscosa	11.5 ± 0.4 c	1.0 ± 0.02 b	4.6 ± 0.1 d	3.1 ± 0.1 c	4.1 ± 0.1 c
A. halimus	27.1 ± 0.03 a	0.6 ± 0.01 d	3.3 ± 0.2 e	6.4 ± 0.1 a	10.2 ± 0.7 b
B. bituminosa	14.1 ± 0.4 b	1.0 ± 0.03 b	10.3 ± 0.3 b	3.2 ± 0.1 bc	0.3 ± 0.01 g
Z. fabago	9.6 ± 0.1 d	1.4 ± 0.02 a	26.8 ± 0.01 a	3.6 ± 0.05 b	18.1 ± 0.04 a
A. donax	13.0 ± 0.2 b	0.7 ± 0.03 c	2.5 ± 0.1 f	1.2 ± 0.04 f	0.2 ± 0.01 h
P. australis	5.8 ± 0.06e	0.3 ± 0.01 e	1.6 ± 0.002 h	2.3 ± 0.01 d	0.6 ± 0.01 e
P. miliaceum	9.7 ± 0.1 d	0.7 ± .010 c	2.2 ± 0.0005g	1.2 ± 0.01 f	0.3 ± 0.003 f
F. vulgare	12.9 ± 0.2 b	0.6 ± 0.03 d	7.7 ± 0.1 c	1.8 ± 0.14 e	2.4 ± 0.12 d
ANOVA	***	***	***	***	***

***: significant at $P< 0.001$. Values followed by the same letter in each column do not differ significantly according to Tukey’s test at $P < 0.05$.

The concentrations of TEs (As, Cd, Pb and Zn) were above the range of concentrations considered as normal for plants (Kabata-Pendias, 2011) in most of the plant species analyzed (Table 3.2). However, these values can be found in plants growing spontaneously in mine affected soils (Martínez-Fernández et al., 2014). The highest TEs concentrations were found in *D. viscosa* (Al, As, Cd, Pb), *P. australis* (As, Cu, Mn and Zn) and *P. miliaceum* (Al, Pb; Table 3.2). The results obtained for *D. viscosa* are close to those found by Barbafieri et al. (2011) and to those reported by Martínez-Fernández (2014) from a pot experiment using soils from the same contaminated area (El Gorguel). The values obtained for As concentration in *P. australis* are within a similar range to those reported by Baroni et al. (2004), while the values of Cu concentration were overall higher than those reported in the literature for this species (7.76-9.05 mg kg^{-1}). Trace element concentration in *P. miliaceum* plants were very similar to those found by Arco-Lázaro et al. (2017) and Párraga-Aguado et al. (2015) in pot experiments using soils from the same mining area. However, the concentrations of As, Pb and Zn in this species were lower than those reported in the general literature (Table 3.3), while Cd and Cu ones were comparable to those found in the aerial parts of this species by Marchiol et al.(2013) and Kabas et al.(2012). The concentrations of TEs and As found in *B. bituminosa* were within similar ranges to those previously reported for this species in soils from this mining area (Arco-Lázaro et al., 2017). Contrastingly, the TEs concentrations in *A. halimus* and *Z. fabago* were much lower than those reported by Pardo et al. (2017) for plants grown in mine tailings with extremely high TEs concentrations. The plants of *F. vulgare* showed overall low TEs concentrations, while *A. donax* presented quite high Zn concentrations (similar to those in *D. viscosa*), which are in agreement with the results of Barbafieri et al. (2011) (Table 3.3).

The plant individuals collected did not show any evident toxicity symptom and were all growing spontaneously in areas heavily affected by mining activity. In fact, with the only exception of Zn in most samples (in particular *D. viscosa*, *A. donax* and *P. australis*; Table 3.2) and As, Cd or Pb in some of them (e.g., *D. viscosa*, *P.australis* and *P. miliaceum*), toxic concentrations of the different TEs were not reached in the studied plants. Critical toxicity levels of TEs in plants are (mg kg^{-1}): As 5-20, Cd 5-30, Cu 20-100, Mn 400-1000, Pb 30-300, Zn 100-400 (Kabata-Pendias, 2011). Positive significant correlations were found between the concentrations of the different TEs in the plans (Table 3.4), which is a common feature of plants growing in metalliferous and TEs contaminated soils (Clemente et al., 2019; Pardo et al., 2016, 2017).

Table 3.2. Trace element concentrations in the plants used in the experiment (average ± se; n = 2).

Plants	**Al (mg kg^{-1})**	**As (mg kg^{-1})**	**Cd (mg kg^{-1})**	**Cu (mg kg^{-1})**	**Fe (mg kg^{-1})**	**Mn (mg kg^{-1})**	**Pb (mg kg^{-1})**	**Zn (mg kg^{-1})**
D. viscosa	563.4 ± 47.0 a	5.6 ± 0.08 a	8.0 ± 0.31 a	13.70 ± 0.12 b	1099.4 ± 29.3 a	70.9 ± 0.2 c	108.5 ± 3.374 a	347.5 ± 2.9 b
A. halimus	76.4 ± 4.7 d	0.7 ± 0.15 c	2.8 ± 0.03 d	5.81 ± 0.17 e	106.1 ± 6.1 fg	73.5 ± 0.9 c	11.1 ± 0.8 e	254.9 ± 6.1 c
B. bituminosa	200.0 ± 1.5 c	1.4 ± 0.10 bc	2.2 ± 0.07 d	7.46 ± 0.05 d	282.4 ± 4.1 d	70.6 ± 2.0 c	45.4 ± 1.7 c	197.7 ± 3.1 d
Z. fabago	88.6 ± 14.6 d	1.9 ± 0.26 b	0.5 ± 0.1 e	8.00 ± 0.18 d	101.6 ± 1.4 g	52.5 ± 0.1 e	5.1 ± 0.3 g	158.2 ± 1.4 e
A. donax	364.2 ± 6.9 b	0.6 ± 0.12 c	4.1 ± 0.06 c	11.19 ± 0.21 c	230.3 ± 4.0 e	84.2 ± 1.4 b	17.8 ± 0.7 d	314.8 ± 3.8 b
P. australis	336.9 ± 4.2 b	3.0± 0.27 ab	5.7 ± 0.16 b	17.74 ± 0.24 a	446.2 ± 9.9 c	241.4 ± 0.4a	77.1 ± 3.4 b	646.0 ± 19.3 a
P. miliaceum	571.7 ± 22.5 a	2.1 ± 0.74 b	5.4 ± 0.05 b	14.46 ± 0.27 b	670.0 ± 8.2 b	59.2 ± 0.5 d	94.1 ±1.9 ab	276.8 ± 1.7 c
F. vulgare	95.7 ±3. 8 cd	1.3 ± 0.03 bc	0.56 ± 0.02 e	5.56 ± 0.1 e	121.2 ± 3.0 f	34.4 ± 0.4 f	8.3 ± 0.364 f	76.3± 2.5 f
ANOVA	***	***	***	***	***	***	***	***

***: significant at $P < 0.001$. Values followed by the same letter in each column do not differ significantly according to Tukey's test at $P < 0.05$.

Table 3.3. Trace elements (TEs) concentration (mg kg^{-1}) in plants of *D. viscosa, A. donax, P. australis* and *P. miliaceum* from different sites affected by mining activities.

TEs	*D. viscosa*	*A. donax*	*P. australis*	*P. miliaceum*	References
As	Leaves 2.97		Leaves 3.71	Leaves 7.5	Baroni et al. (2004)
Cd	Leaves 10-44 Stems 5-17 Roots 5-19	0.5-3.0			Barbafieri et al. (2011)
	Leaves 2.97				Baroni et al. (2004)
	Shoots 122 Roots 106			Leaves/stems 7.76 Roots 417	Marchiol et al. (2013)
	Shoots 1.06 Roots 5.66				Conesa et al. (2006)
Cu	Shoots 55.9 Roots 44.7		Shoots/stems 7.76 Roots 9.05	Shoots 5.22 Roots 724	Marchiol et al. (2013)
	Shoots 19 Roots 5.6				Conesa et al. (2006)
				Shoots 19.7 Roots 1.61	Kabas et al. (2012)
Pb	Leaves 230-700 Stems 56-150 Roots 34-430	Leaves/Stems 10-38 Root 8-21			Barbafieri et al. (2011)
	Roots 569				Marchiol et al. (2013)
				Shoots 20.4 Roots 350	Kabas et al. (2012)
				Shoots 981	Melendo et al. (2002)
Zn	Leaves 770-2900 Stems 210-430 Root 200-370	Leaves/stems 210-630 Root 130-240			Barbafieri et al. (2011)
	Roots 1172				Marchiol et al. (2013)
	Leaves 2000				Jiménez-Cárceles et al. (2008)
	Shoots 638				Conesa et al. (2006)
				Shoots 520 Roots 662	Kabas et al. (2012)

Table 3.4. Pearson's correlation coefficients between the concentrations of the different TEs in the plants (n = 8).

	Al Plants	**As Plants**	**Cd Plants**	**Cu Plants**	**Fe Plants**	**Mn Plants**
CdPlants	0.887**	0.728*				
CuPlants	0.796*	0.588	0.818*			
FePlants	0.885**	0.887**	0.890**	0.672		
PbPlants	0.883**	0.795*	0.885**	0.799*	0.934**	0.339
ZnPlants	0.467	0.413	0.707	0.852**	0.403	0.934**

* and **: significant at $P < 0.05$ and 0.01, respectively

Lignin is one of the main constituents of plants, found between the cell walls of the fibers and performs the function of binder by imparting hardness and rigidity to the plant (Zimbardi et al., 1999). Lignin usually represents 15-35 % of plant biomass, depending on the species. In the present experiment, the highest lignin concentrations were found in *D. viscosa* (27.0 %) and *A. halimus* (22.4 %), while the other species showed lower and similar concentrations (Table 3.5). Comparing the experimental data with those of plant species used for bioenergy production (e.g., *Sorghum vulgare* Pers. (stalk) 18.69 %, *Arundo donax* L. (stems) 16.8 %, *Miscanthus sinensis* var. *Giganteus* (stems) 12.8 %, *Panicum virgatum* L. (whole plant) 17.6 %; Cosentino et al., 2007; Palumbo and Mastro, 2014; Pimentel et al. ,2017), our results here were generally higher than in those species. The content of lignin of each species is of great relevance for the use of plant biomass for energy purposes, for example, it may condition its suitability for combustion. Regarding soluble carbohydrates, the highest values were found in *A. donax* and *F. vulgare* (Table 3.5), which indicates the feasibility for microbial degradation; while the concentration of holocellulose (sum of cellulose and hemicellulose) was quite similar in all samples and only slightly higher for *P. miliaceum* and *P. australis*. The values found for *A. donax* were similar to those found by Cencič et al. (2007) for this species, and for the rest of the species the concentrations determined were generally higher than those found in other lignocellulosic species (53-65 %; see Chapter 1, Table 1.5).

Volatile solids (VS) concentrations were high for all species with values ranging from 80.6 to 93.7 % (Table 3.5), close to the values found for *P. miliaceum* 87.2 %, *S. marianum* 77.7 %, *H. annuus* 83.2 % and *N. glauca* 78.1 % (Bernal et al., 2019). The concentration of VS indicates the quantity of organic matter contained in the biomass that can be potentially transformable into biogas, or combusted and is therefore another parameter of relevance regarding the possible use of the different species for bioenergy production.

The elemental (C, N, S and H) composition of the plants is shown in Table 3.6. The concentrations of C (%) were very similar in all samples, while N ones were significantly higher in the leguminous species (*Z. fabago* and *B. bituminosa*) and in *A. halimus* than in the rest of the plants. Sulfur concentration was also the highest in *Z. fabago* samples. These results were used to determine the HHV, which were found within a rather narrow range (15.66-18.75 MJ kg^{-1}), the lowest values being found in *Z. fabago* and the highest in *A. donax* samples (Table 3.6). These values are in the upper limit of the range found by Bernal et al. (2019) for *P. miliaceum*, *S. marianum*, *H. annuus* and *N. glauca* (14.76-17.45 MJ kg^{-1}). The results of the present experiment were slightly higher than those determined for *S. marianum* and *H. annuus* plants using a calorimetric bomb (12.5-17.5 MJ kg^{-1}) (Hunce et al., 2019). Results of McKendry (2002) for different types of plants (fir 21 MJ kg^{-1}, Danish pine 21.2 MJ kg^{-1}, willow 20 MJ kg^{-1}, poplar 18.5 MJ kg^{-1}, cereal straw 17.3 MJ kg^{-1}, Switchgrass 17.4 MJ kg^{-1}, Miscanthus 18.5 MJ kg^{-1}) and by Boundy et al. (2011) for herbaceous species (17.2 MJ kg^{-1}) were close to most values found here. The HHVs found were lower than that of coal (22.7 MJ kg^{-1}; Boundy et al., 2011), but close to the values of forest residues and farmed trees (15.4 and 19.5 MJ kg^{-1}, respectively; Boundy et al., 2011). Even though the lignin content of lignocellulosic biomass has been generally related with the heating value (Saidur et al., 2011), no significant correlation was found between lignin concentration and HHV in our samples. However, the content of VS and carbohydrates showed significant positive correlations with HHVs in the plants studied (r = 0.946 and 0.731, $P < 0.001$ and 0.05, respectively). The calorific value did not seem to be influenced by the presence of TE concentrations in the plant biomass, as there were no significant correlations between As, Cd, Cu, Pb and Zn concentrations and HHV in the samples (data not shown). In fact, Mg and Na concentrations in the plants negatively correlated HHV (r = -0.765 and -0.914, $P < 0.05$ and 0.01, respectively), indicating that the salt accumulation in the plants can affect their calorific potential and therefore the use of the plants for combustion. High mineral content and high ash values can result in low HHV and such a high ash content may also represent a potential problem of fouling and slagging in combustion equipment (Bernal et al., 2019).

3.2. Anaerobic digestion

The biogas produced after 16 days of anaerobic digestion (B_m) was the lowest for *A. halimus* followed by *Z. fabago* and *D. viscosa*, without significant differences between the rest of the plants (*P. miliaceum, A. donax, P. australis, B. bituminosa* and *F. vulgare*; Table 3.7). In general, the values found were lower than those reported for other energy crops such as sunflower (up to 454 mL g^{-1} VS; Amon et al., 2007). However, a lag-phase was found for *P. miliaceum* at the beginning of the experiment (Figure 3.1), indicating an initial difficulty for the microbial degradation. The composition of the plants cannot explain this behavior, as none of the studied factors differed strongly from those of the other plant species. In fact, the B_m values were the highest at the end of the experiment, then the initial lag-phase of microbial degradation did not limit the anaerobic process of biogas production. Similar results were found by Bernal et al. (2019) for this plant species (B_m 235-270 mL g^{-1} SV).

The results fitted at highly significant level the first order kinetic model (at $P < 0.001$ in all samples; data do not shown). The values of B_0 mirrored those of B_m and followed a very similar order for the different species studied: the highest for *A. donax* and *P. australis* without significant differences with the results for *B. bituminosa*, *P. miliaceum* and *F. vulgare*; and lowest for *A. halimus* (Table 3.7). Therefore, the anaerobic degradation of the plant biomass was almost complete at the end of the experimental time. The highest value of the rate constant (K_d) was found for *Z. fabago* (Table 3.7), indicating that the maximum production of biogas was reached earlier for this biomass than for the other plant species, regardless of the total amount of biogas produced. This could be attributed to the lower amount of lignin in *Z. fabago* plants compared to the other species, as it has been previously shown that the difficulty of degradation of highly lignocellulosic materials leads to limitations during the hydrolysis phase (Bernal et al., 2019; Hunce et al., 2019).

Table 3.5. Chemical composition of the plant samples used in the experiments (average values ± se, n = 2).

Plants	**Ash (%)**	**VS (%)**	**Holocellulose (%)**	**Lignin (%)**	**Soluble carbohydrates (g kg^{-1} dw)**
D. viscosa	7.03 ± 0.02 c	90.82 ± 0.16 b	50.94 ± 7.21 bc	26.96 ± 0.72 a	54.85 ± 2.95 bc
A. halimus	14.25 ± 0.14 b	84.14 ± 0.05 c	62.11 ± 5.73 abc	22.40 ± 0.23 b	12.40 ± 0.40 f
B. bituminosa	5.31 ± 0.06 c	93.19 ± 0.02 a	65.19 ± 0.57 abc	20.09 ± 0.45 bc	26.5 ± 0.80 ef
Z. fabago	16.28 ± 0.31 a	80.57 ± 0.57 d	48.58 ± 6.07 c	17.08 ± 0.92 c	35.15 ± 0.95 de
A. donax	6.26 ± 0.19 c	93.74 ± 0.19 a	68.23 ± 0.59 abc	20.92 ± 1.67 bc	83.85 ± 3.15 a
P. australis	6.81 ± 0.45 c	93.19 ± 0.45 a	72.32 ± 056 a	20.87 ± 0.55 bc	49.5 ± 0.01 cd
P. miliaceum	7.09 ± 0.61 c	92.92 ± 0.60 a	72.42 ± 0.65 a	18.42 ± 0.33 bc	44.15 ± 3.05 cde
F. vulgare	6.92 ± 0.33 c	93.08 ± 0.33 a	70.98 ± 0.38 ab	19.59 ± 0.33 bc	69.35 ± 7.35 ab
ANOVA	***	***	**	**	***

** and ***: significant at $P < 0.01$ and 0.001, respectively. Values followed by the same letter in each column do not differ significantly according to Tukey’s test at $P < 0.05$.

Table 3.6. Elemental composition and higher heating value (HHV) of the plants (average values ± se; n = 2).

Plants	**C (%)**	**N (%)**	**C/N**	**S (%)**	**H (%)**	**HHV (MJ kg^{-1})**
D. viscosa	46.24 ± 0.09 a	0.57 ± 0.01 e	80.85 ± 1.44 a	0.10 ± 0.02 d	5.12 ± 0.08 d	17.96 ± 0.03 c
A. halimus	41.33 ± 0.01 e	1.21 ± 0.00 b	34.27 ± 0.07 d	0.14 ± 0.02 cd	4.66 ± 0.07 e	16.03 ± 0.04 e
B. bituminosa	45.01 ± 0.09 b	1.48 ± 0.01 ab	30.49 ± 0.26 d	0.07 ± 0.01 d	5.07 ± 0.02 d	17.62 ± 0.04 d
Z. fabago	40.33 ± 0.02 f	1.99 ± 0.08 a	20.35 ± 0.86 e	0.52 ± 0.07 a	4.75 ± 0.02 e	15.66 ± 0.01 f
A. donax	43.13 ± 0.02 c	0.72 ± 0.02 cd	59.93 ± 1.22 bc	0.32 ± 0.02 b	7.50 ± 0.01 a	18.75 ± 0.00 a
P. australis	43.38 ± 0.05 c	0.59 ± 0.00 e	73.84 ± 0.27 a	0.29 ± 0.02 bc	7.02 ± 0.01 b	18.49 ± 0.01 b
P. miliaceum	42.70 ± 0.04 d	0.66 ± 0.03 d	64.43 ± 2.75 b	0.19 ± 0.00 bcd	6.38 ± 0.03 c	17.87 ± 0.03 c
F. vulgare	43.44 ± 0.11 c	0.77 ± 0.02 c	56.38 ± 1.39 c	0.08 ± 0.02 d	6.11 ± 0.07 c	17.89 ± 0.08 c
ANOVA	***	***	***	***	***	***

***: significant at $P < 0.001$. Values followed by the same letter in each column do not differ significantly according to Tukey's test at $P < 0.05$.

Biogas production and biogas production potential obtained in the anaerobic digestion experiment were generally low for all plant species compared to those reported for energy crops such as maize (345 mL g^{-1} in whole plant), and also to other herbaceous species, such as nettle (210-420 mL g^{-1}), ryegrass (360 mL g^{-1}) or sunflower (454 mL g^{-1} in whole plant) (Amon et al., 2007; Braun et al., 2007; Raposo et al., 2011). Indeed, only the values of B_0 for *P. miliaceum* and *A. donax* were close to those previously reported by Bernal et al. (2019) (Table 3.7).

The variability of values of the biogas production potential (and biochemical methane potential, BMP) of crops may be due to the different lignin content (Gunaseeland, 2007; Nizami et al., 2009) and to the physiological state of the plants at the time of the harvest. Actually, the biogas yield (and BMP) decreases in the late harvesting times (Braun et al., 2007). Also, B_0 depends on total soluble carbohydrates, concentrations of N, ash and the ratio lignin to ADF (acid detergent fiber) (Gunaseelan, 2007). In fact, the data for biogas production potential of *P. miliaceum* can be linked to its high concentration of N and low C/N ratio.

The BMP values were higher for *A. donax* (227.4 mL g^{-1}), *P. australis* (218.0 mL g^{-1}) and *P. miliaceum* (204.6 mL g^{-1}) species (Table 3.7), with values close to those found for solid wastes derived from *H. annuus* oil extraction (between 107 and 227 mL g^{-1}; Raposo et al. (2008), and of maize, thistle and sorghum silage (267, 308, and 241 mL g^{-1}, respectively; Kalamaras and Kotsopoulos, 2014). However, the values obtained from the aerial vegetative parts of *S. marianum* (174 mL g^{-1}) and *H. annuus* (119 mL g^{-1}) by Hunce et al. (2019) can be compared with the lowest results obtained in this study.

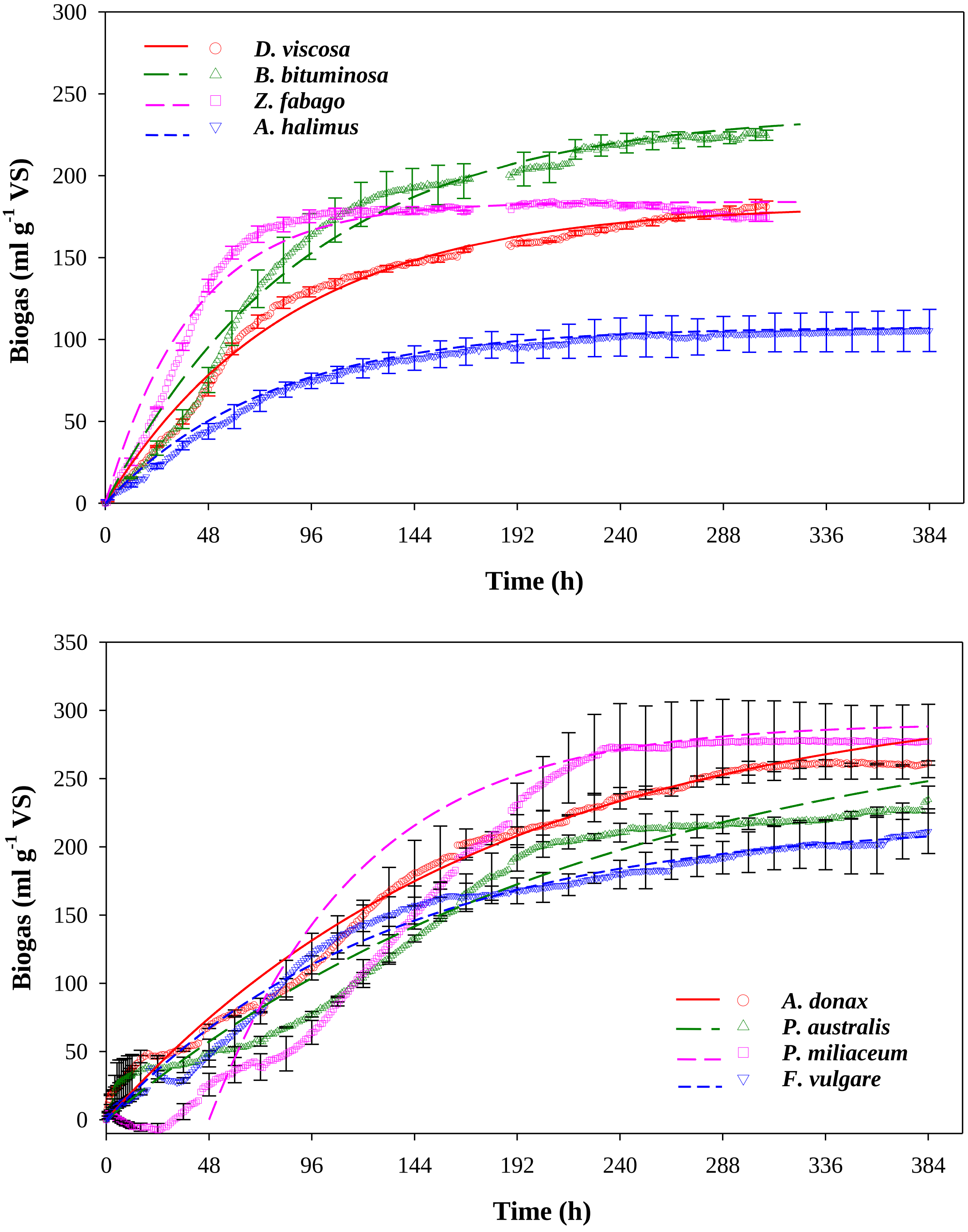

Figure 3.1. Biogas production from the plants under anaerobic conditions. The symbols are the experimental data and the lines the degradation predicted by a first-order kinetic model for each sample (n = 2).

The theoretical BMP (TBMP) had values between 497.9 mL g^{-1} (*D. viscosa*) and 576.5 mL g^{-1} (*A. halimus*), giving an average anaerobic biodegradability between 15.1 % (*A. halimus*) and 43.9 % (*A. donax*). Only the results obtained for *A. halimus*, *Z. fabago*, *D. viscosa* and *F. vulgare* (Table 3.7) were lower than the results found for cuts of wood and hedges and wild plants (32.7-44.9 %), as well as for crops (Triolo et al., 2012), while similar or even higher values were obtained for the rest of species (Table 3.7). The low degradability found for *A. halimus* and *D. viscosa* can be associated with the higher lignin value obtained for the same species compared to the others (22.4 and 27.0 %, respectively). It is known that lignin influences the production of biogas in the anaerobic digestion of lignocellulosic substrates such as agricultural crops, due to its low biodegradability associated to its complex structure (Buffiere et al., 2006). But other factors, such as the salt content, may have reduced their anaerobic degradability, especially in *A. halimus*, with high Na concentration in their biomass. Like other halophytes, *A. halimus* accumulates Na^+ ions in its tissues for osmotic adjustment as a tolerance mechanism in response to soil salinity (Walker and Lutts, 2014). In our experiment, negative correlations were found between the concentration of Mg and Na and BMP and anaerobic biodegradability (for Mg: r = -0.926 and -0.878 both at $P < 0.01$, respectively; for Na: r = -0.715 and -0.707 at $P < 0.05$, respectively). Contrastingly, *Kd* positively correlated with P, Ca and Na concentrations in the plants (r = 0.832, 0.809 and 0.800, respectively, all $P < 0.05$), indicating a faster degradation of the plants with the highest concentrations of these elements.

The concentrations of VS, H, and HHV, correlated positively with B_m, B_0 and BMP values in the plants (Table 3.8). However, no significant correlations were found between anaerobic digestion parameters and holocellulose, lignin or carbohydrates concentrations in the plants, which indicates that other factors other than these influenced the anaerobic degradation, such as the mineral content (Mg and Na) previously discussed, which also affected the thermal energy released by combustion (HHV).

Table 3.7. Results of anaerobic degradation of the plants and the parameters of the first-order kinetic model (mean ± se; n = 2).

Plants	**B_m (mL g^{-1}SV)**	**B_0 (mL g^{-1}SV)**	***Kd* (h^{-1})**	**BMP (mL CH_4g^{-1} SV)**	**TBMP (mL CH_4g^{-1} SV)**	**Anaerobic biodegradability**
D. viscosa	180.9 ± 3.6 bc	182.2 ± 5.0 bc	0.012 ± 0.001 bc	131.2±3.6bc	497.9 ± 26.6	26.4± 0.7bcd
A. halimus	116.6 ± 10.7 c	119.9 ± 14.8 c	0.012 ± 0.004 bc	86.0±10.4 c	576.5± 30.6	15.1 ± 2.9 c
B. bituminosa	224.4 ± 3.2 ab	239.2 ± 0.6 ab	0.011 ± 0.001 bc	177.6±1.1 ab	525.3 ± 6.2	33.8 ± 0.2abc
Z. fabago	173.8 ± 2.5 bc	184.0 ± 0.1 bc	0.025 ± 0.001 a	130.6±0.04bc	514.7± 39.9	25.5 ± 2.0cd
A. donax	261.4 ± 1.5 a	315.9 ± 25.5 a	0.006 ± 0.001 bc	227.4±18.4a	517.1± 9.8	43.9 ± 2.7 a
P. australis	234.5 ± 9.6 ab	307.0 ± 23.6 a	0.004 ± 0.001 c	218.0±16.8 a	533.5± 9.3	40.8± 2.4ab
P. miliaceum	277.5 ± 26.9 a	292.3 ± 38.8 ab	0.015 ± 0.003 ab	204.6±27.2 ab	518.9± 2.7	39.5± 5.5 abc
F. vulgare	211.5 ± 16.1 ab	219.4 ±15.3 abc	0.008 ±0.0002 bc	154.6±108abc	524.3± 2.0	29.5± 2.2abcd
ANOVA	***	**	**	**	**Ns**	**

** and ***: significant at P < 0.01 and 0.001, respectively. Ns = not significant. Values followed by the same letter in each column do not differ significantly according to Tukey's test at $P < 0.05$.

Table 3.8. Pearson's correlation coefficients between plant composition, HHV and anaerobic digestion related parameters (n = 8).

	Ashes	**VS**	**N**	**H**	**HHV**	**B_m**	**B_0**	***Kd***	**BMP**
B_m	-0.721 *	0.759*	-0.448	0.789*	0.760*				
B_0	-0.675	0.730*	-0.471	0.896**	0.792*	0.947***			
Kd	0.721*	-0.777*	0.739*	-0.658	-0.792*	-0.336	-0.476		
BMP	-0.693	0.740*	-0.451	0.882**	0.798*	0.943***	0.998***	-0.488	
Anaerobic Biodegradability	-0.700	0.738*	-0.451	0.869**	0.016	0.955***	0.994***	-0.451	0.996***

*, ** and ***: significant at P < 0.05, 0.01 and 0.001, respectively

Although high lignin concentration in the plants may be indicative of a low biodegradability (Bernal et al., 2009; Nizami et al., 2009), the species studied in the present experiment showed lignin concentrations within a rather narrow range (17-27 %), which provoked that this parameter was not significantly related to any of the calorific or biogas determined parameters. Other parameters like VS, Mg and Na concentration in the plants and carbohydrates content (for HHV) seemed to be of higher relevance for the potential of the biomass of the different plant species studied for their energetic transformation.

The results obtained from the correlation test showed that the anaerobic degradation was not influenced by the presence of TEs in the plants (no significant correlations between anaerobic digestion parameters and TEs concentrations in the plants; data not shown). Although Pb accumulation negatively affected the anaerobic degradation of *N. glauca* biomass (225-231 mg Pb kg^{-1} dw), and therefore the biogas production (Bernal et al., 2019), the concentration of Pb in the studied plants did not reach such levels to affect negatively the anaerobic degradation of the plant biomass. All microbial processes require nutrients and certain TEs for their development. Some of these are essential macro- and micronutrients (N, P, S, K, Mg, Na, Ca, Fe, Cr, Co, Cu, Mn, Mo, Ni, Se, V and Zn), which are needed as part of the active site of enzymes (Kida et al., 2001). Some TEs have shown positive effects on anaerobic digestion (Demirel et al., 2011); however, above certain concentrations they become inhibitory or toxic (Zhang et al., 2003). Bozym et al. (2015) reported the TE concentrations in the plants that can be considered toxic for anaerobic digestion: Cd 180, Cu 40, Zn 100 and Pb 30 mg kg^{-1}. The concentrations of TEs determined in the plants were all lower than those values except for Zn (Table 3.2). Probably, the contribution of certain micronutrients, such as Fe and Mn could have stimulated positive effects on the growth of almost all types of microorganisms in the anaerobic digesters (Mudhoo and Kumar, 2013).

7. CONCLUSIONS

The concentration of TEs in the plants from mining sites did not affect biogas production, or the production of thermal energy according to the higher heating value.

In particular, the species *P. miliaceum*, *P. australis* and *A. donax* are the species with the best perspectives for the production of biogas. Contrastingly, *D. viscosa* and *A. halimus* plants have more adequate characteristics for their direct combustion, due to their low ash and humidity values and higher lignin concentrations, than for their anaerobic transformation. However, the presence of elevated Na and Mg concentrations in *A. halimus* can limit its usefulness for combustion. Therefore, the most suitable species for the production of biogas seem to be those belonging to the Poaceae family, and the presence of high Mg and Na concentrations in the plants (aerial parts) can condition the production of energy by anaerobic digestion. Furthermore, the positive correlations between the values of HHV and biogas production suggest that the species investigated can be used for the phytostabilization of TEs contaminated soils, and their biomass used for the production of thermal energy or biogas.

REFERENCES

Amon, T., Amon, B., Kryvoruchko, V., Machmüller, A., Hopfner-Sixt, K., Bodiroza, V., Hrbek, R., Friedel, J., Pötsch, E., Wagentristl, H., Schreiner, M., Zollitsch, W. (2007). Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations, *Bioresource Technology, 98*, 3204–3212.

Arco-Lázaro, E., Martínez-Fernández, D., Bernal, M. P., & Clemente, R. (2017). Response of *Piptatherum miliaceum* to co-culture with a legume species for the phytostabilisation of trace elements contaminated soils. *Journal of Soils and Sediments*, *17*(5), 1349–1357. https://doi.org/10.1007/s11368-015-1261-9.

Barbafieri, M., Dadea, C., Tassi, E., Bretzel, F., Fanfani, L. (2011). Uptake of heavy metals by native species growing in a mining area in Sardinia, Italy: discovering native flora for phytoremediation. *Internal Journal of Phytoremediation, 13*, 985–997.

Barker, A.V., Pilbeam, D.J. (2015). Handbook of Plant Nutrition. (2nd ed.). Boca Raton: CRC Press.

Baroni, F., Boscagli, A., Di Lella, L.A., Protano, G., Riccobono, F. (2004). Arsenic in soil and vegetation of contaminated areas in southern Tuscany (Italy). *Journal of. Geochemical Exploration, 81*, 1–14.

Bernal, M. P., Clemente, R., & Walker, D. J. (2009). Interactions of heavy metals with soil organic matter in relation to phytoremediation. In: Navarro-Aviño, J.P. (Ed.), *Phytoremediation: the Green Salvation of the World*. pp. 109–129. Kerala, India: Research Signpost.

Bernal, M. P., Gómez, X., Chang, R., Arco-Lázaro, E., & Clemente, R. (2019). Strategies for the use of plant biomass obtained in the phytostabilisation of trace-element-contaminated soils. *Biomass & Bioenergy, 126,* 220–230. https://doi.org/10.1016/j.biombioe.2019.05.017.

Boundy, B., Diegel, S.W., Wright, L., Davis, S.C. (2011). Biomass Energy Data Book, 4th ed. Oak Ridge National Laboratory, Oak Ridge, US Department of Energy, Tennessee (accessed 23 January 2019). http://ledsgp.org/wp-content/uploads/2015/10/ biomass-energy-data-book.pdf.

Bozym, M., Florczak, I., Zdanowska, P., Wojdalski, J., & Klimkiewicz, M. (2015). An analysis of metal concentrations in food wastes for biogas production. *Renewable Energy, 77,* 467–472. https://doi.org/10.1016/j.renene.2014.11.010

Braun, R. (2007). Anaerobic digestion - a multi-faceted process for energy, environmental management and rural development, In: P. Ranalli (Ed.), *Improvement of Crop Plants for Industrial End Users*, pp. 335–416. Dordrecht: Springer.

Buffiere, P., Loisel, D., Bernet, N., Delgenes, J.P. (2006). Towards new indicators for the prediction of solid waste anaerobic digestion properties. *Water Science & Technology, 53,* 233–241. https://doi.org/10.2166/wst.2006.254.

Cencič, V., Jodice, R., Migliardi, D., Picco, D., Pin, M., Tomasinsig, E., Zanotto, G., Radu, R., Taccani, R., Chindaruska, S., Barbieri, S., Bianco, D., Michelutti, R., Monaco, B. (2007). I biocarburanti. *Le filiere produttive, le tecnologie, i vantaggiambientalie le prospettivedi diffusione.* :, Volume 27, pp. 26-158. Padriciano, Italy: Area Science Park.

Clemente, R., Arco-Lázaro, E., Pardo, T., Martín, I., Sánchez-Guerrero, A., Sevilla, F., & Bernal, M. P. (2019). Combination of soil organic and inorganic amendments helps plants overcome trace element induced oxidative stress and allows phytostabilisation. *Chemosphere, 223,* 223–231. https://doi.org/10.1016/j.chemosphere.2019.02.056

Conesa, H.M. (2003). Informe Agronómico sobre la finca de ‘‘Las Jacobas’’. Technical Report. Universidad Politécnica de Cartagena, Cartagena, Spain.

Conesa, H.M., Faz, A., Arnaldos, R. (2006) Heavy metal accumulation and tolerance in plants from mine tailings of the semiarid Cartagena- La Union Mining District (SE Spain). *Science of the Total Environment, 366,* 1–11.

Conesa, H.M., Robinson, B.H., Schulin, R., Nowack, B. (2008a) Metal extractability in acidic and neutral mine tailingsfrom the Cartagena—La Union Mining District (SE Spain). *Applied Geochemistry, 23,* 1232–1240.

Conesa, H.M., Schulin, R., Nowack, B. (2008b) Mining landscape: A cultural tourist opportunity or an environmental problem? The study case of the Cartagena–La Unión Mining District (SE Spain). *Ecological Economics, 64,* 690–700.

Conesa, H.M, & Faz, A. (2009). Metal Uptake by Naturally Occurring Vegetation in a Metal Polluted Salt Marsh in Southeast Spain. In: A. Faz- Cano, A. R. Mermut, J. M. Arocena and R. Ortiz (Eds.), Land Degradation and Rehabilitation (Advances in Geoecology, 40), pp. 287-294. Germany: Catena- Verlag.

Conesa, H. M., & Schulin, R. (2010). The Cartagena-La Unión mining district (SE Spain): A review of environmental problems and emerging phytoremediation solutions after fifteen years research. *Journal of Environmental Monitoring, 12,* 1225–1233 |https://doi.org/10.1039/c000346h.

Conesa, H.M., J. A. Pérez-Chacon, J.A., Arnaldos, R., Moreno-Caselles, J., Faz-Cano, A. (2010). In situ heavy metal accumulation in lettuce growing near a former mining waste disposal area: Implications for agricultural management. *Water, Air and Soil Pollution*, *208*, 377-383.

Cosentino, S.L.; Sanzone, E.; Bellomia, L.; Sortino, O.; Belligno, A. (2007). Chemical composition and energetic value of perennial ligno-cellulosic species (Miscantus x Giganteus Greef and Deu. and *Arundo donax* L.) grown in Mediterranean region. In Proceedings of the 15th European Biomass Conference & Exhibition, Berlin, Germany, 7–11 May 2007.

Palumbo, A.; Mastro, G. Produzione di "bioetanolo di seconda generazione" dal cardo (*Cynara cardunculus* L. var. altilis DC.). In Proceedings of the SIA—Società Italiana di Agronomia, XLIII Convegno Nazionale, Pisa, Italy, 17–19 September 2014.

Deheyn, D., Jangoux, M., & Warnau, M. (2000). Alteration of bioluminescence in A*mphipholis squamata* (Ophiuroidea: Echinodermata) by heavy metals contamination: a field study. *Science of the Total Environment, 247*, 41-49. https://doi.org/10.1016/S0048-9697(99)00465-9.

Demirel, B., Scherer, P. (2011). Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. *Biomass & Bioenergy, 35*, 992-998. https://doi.org/10.1016/j.biombioe.2010.12.022.

Domínguez, M.T., Madejón, P., Madejón, E., Diaz, M.J. (2017a). Novel energy crops for Mediterranean contaminated lands: valorization of *Dittrichia viscosa* and *Silybum marianum* biomass by pyrolysis. *Chemosphere, 186*, 968–976. https://doi.org/10. 1016/j.chemosphere.2017.08.063.

Domínguez, M.T., Montiel-Rozas, M.M., Madejón, P., Diaz, M.J., Madejón, E. (2017b). The potential of native species as bioenergy crops on trace-element contaminated Mediterranean lands. *Sci. Total Environ*. 590–591, 29–39. https://doi.org/10.1016/j. scitotenv.2017.03.018.

EPA (2001). Method 1684. *Total, Fixed and Volatile Solids in Water, Solids, and Biosolids*. Washington: US Environmental Protection Agency.

Fanfani, L., Caboi, R., Cidu, R., Cristini, A., Frau, F., Lattanzi, P., Zuddas, P. (2000). Impatto ambientale dell'attività mineraria in Sardegna: studi mineralogici e geochimici. *Rendiconti Seminario Facoltà Scienze Università Cagliari Supplemento, 70*, 249-264.

Grippi, D., Clemente, R., & Bernal, M.P. (2020). Chemical and bioenergetic characterization of biofuels from plant biomass: Perspectives for Southern Europe. *Applied Scence,10*(10) 3571. https://doi.org/10.3390/app10103571.

Gunaseelan, V.N. (2007). Regression models of ultimate methane yields of fruits and vegetable solid wastes, sorghum and napiergrass on chemical composition, *Bioresource Technology, 98,* 1270–1277.

Hansen, T. L., Schmidt, J. E., Angelidaki, I., Marca, E., Jansen, J. L. C., Mosbæk, H., & Christensen, T. H. (2004). Method for determination of methane potentials of solid organic waste. *Waste Management, 24,* 393–400. https://doi.org/10.1016/j.wasman.2003.09.009.

Hedge, J. E., Hofreiter, B.T. (1962) *Carbohydrate Chemistry*, pp. 17. Whistler, R. L. and Be Miller, J. N.(Eds.). New York: Academic Press.

Hunce, S. Y., Clemente, R., & Bernal, M. P. (2019). Energy production potential of phytoremediation plant biomass: *Helianthus annuus* and *Silybum marianum*. *Industrial Crops an Products , 135*, 206–216. https://doi.org/10.1016/j.indcrop.2019.04.029.

Jiménez-Cárceles, F.J.; Álvarez-Rogel, J.; Conesa-Alcaraz, H. (2008). Trace element concentrations in saltmarsh soils strongly affected by wastes from metal sulphide mining areas. *Water, Air and Soil Pollution*, *188,* 283-295.

Kabata-Pendias, A. (2011). Trace Elements in Soils and Plants, (4th ed.). USA: CRC Press.

Kabas, S., Faz, A., Acosta, J.A., Zornoza, R., Martínez-Martínez, S., Carmona, D.M., Bech, J. (2012). Effect of marble waste and pig slurry on the growth of native vegetation and heavy metal mobility in a mine tailing pond. *Journal of Geochemical Exploration*, *123*, 69-76. http://dx.doi.org/10.1016/j.gexplo.2012.07.008

Kalamaras, S.D., Kotsopoulos, T.A. (2014). Anaerobic co-digestion of cattle manure and alternative crops for the substitution of maize in South Europe. *Bioresource Technol*ogy, *172*, 68–75. https://doi.org/10.1016/j.biortech.2014.09.005.

Kida, K., Shigematsu, T., Kijima, J., Numaguchi, M., Mochinaga, Y., Abe, N. (2001). Influence of Ni^{2+} and Co^{2+} on methanogenic activity and the amounts of coenzymes involved in methanogenesis. *Journal of Bioscience and Bioengineering, 91*, 590-595.

Marchiol, L., Fellet, G., Boscutti, F., Montella, C., Mozzi, R., & Guarino, C. (2013). Gentle remediation at the former "Pertusola Sud" zinc smelter: Evaluation of native species for phytoremediation purposes. *Ecological Engineering, 53,* 343–353. https://doi.org/10.1016/j.ecoleng.2012.12.072

María-Cervantes, A., Jiménez-Cárceles, F.J. & Álvarez-Rogel, J. (2009). As, Cd, Cu, Mn, Pb, and Zn contents in sediments and mollusks (*Hexaplex trunculus* and *Tapes decussatus*) from coastal zones of a Mediterranean Lagoon (Mar Menor, SE Spain) affected by mining wastes. *Water, Air and Soil Pollution, 200*, 289–304. https://doi.org/10.1007/s11270-008-9913-7.

Martínez-Fernández, D., Arco-Lázaro, E., Bernal, M. P. & Clemente, R. (2014). Comparison of compost and humic fertiliser effects on growth and trace elements accumulation of native plant species in a mine soil phytorestoration experiment. *Ecological Engineering*, *73*, 588–597. https://doi.org/10.1016/j.ecoleng.2014.09.105

McKendry, P. (2002). Energy production from biomass (Part 1): overview of biomass. *Bioresource Technol*ogy, *83,* 37–46.

Melendo, M., Benítez, E., Nogales, R. (2002). Assessment of the feasibility of endogeneous Mediterranean species for phytoremediation of Pb-contaminated areas. *Fresenius Environmental Bull*etin, *11*, 1105–1109.

Mendez, M.O., Maier, R.M. (2008) Phytostabilization of mine tailings in arid and semiarid environments—an emerging remediation technology. *Environmental Health Perspective*, *116*, 278–283.

Mudhoo, A., Kumar, S., (2013). Effects of heavy metals as stress factors on anaerobic digestion processes and biogas production from biomass. *International Journal of Environmental Science, 10,* 1383–1398. https://doi.org/10.1007/s13762-012-0167-y.

Navarro, M.C., Perez-Sirvent, C., Martinez-Sanchez, M.J., Vidal, J., Marimon, J. (2006). Lead, cadmium and arsenic bioavailability in the abandoned mine site of Cabezo Rajao (Murcia, SE Spain). *Chemosphere*, *63*, 484–489.

Nizami, A., Korres, N.E., Murphy, J.D. (2009). Review of the integrated process for the production of grass biomethane. *Environmental Science & Technology, 43*, 8496–8508. https://doi. org/10.1021/es901533j.

Pardo, T., Bes, C., Bernal, M.P., Clemente, R. (2016). Alleviation of environmental risks associated to severely contaminated mine tailings using amendments: modelling of trace elements speciation, solubility and plant accumulation. *Environmental Toxicology and Chemistry, 35*, 2874-2884.

Pardo, T., Bernal, M.P., Clemente, R. (2017). Phytostabilisation of severely contaminated mine tailings using halophytes and field addition of organic and inorganic amendments. *Chemosphere,178*, 556-564.

Párraga-Aguado, I., González-Alcaraz, M.N., Schulin, R., Conesa, H.M. (2015). The potential use of *Piptatherum miliaceum* for the phytomanagement of mine tailings in semiarid areas: role of soil fertility and plant competition. *Journal of Environmental Management, 158*, 74-84.

Pimentel, L.D., Batista, V.A.P., de Barros, A.F., Teófilo, R.F., dos Santos Dias, L.A. (2017). Caracterização química e bioenergética de grupos agronômicos de sorgo. *Pesquisa Agropecuária Tropical , 47*, 424–431

Raposo, F., Borja, R., Rincon, B., Jimenez, A.M. (2008). Assessment of process control parameters in the biochemical methane potential of sunflower oil cake. *Biomass & Bioenergy, 32*, 1235–1244. https://doi.org/10.1016/j.biombioe.2008.02.019.

Raposo, F., De la Rubia, M.A., Fernández-Cegrí, V., Borja, R. (2011). Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures, *Renewable & Sustainable Energy Reviews, 16*, 861–877.

Sanchiz, C., García-Carrascosa, A.M., Pastor, A. (2000). Heavy metal contents in soft-bottom marine macrophytes and sediments along the Mediterranean coast of Spain. *Marine Ecology, 21*, 1–16.

Saidur, R., Abdelaziz, E.A., Demirbas, A., Hossain, M.S., Mekhilef, S. (2011). A review on biomass as a fuel for boilers. *Renewable & Sustainable Energy Reviews, 15*, 2262–2289. https:// doi.org/10.1016/j.rser.2011.02.015.

Sathya, A., Vijayabharathi, R., Gopalakrishnan, S. (2016). Soil microbes: the invisible managers of soil fertility. In: Singh D., Singh H., Prabha R. (Eds) *Microbial Inoculants in Sustainable Agricultural Productivity*, pp. 1-16. New Delhi: Springer.

Sheng, C., & Azevedo, J.L.T. (2005). Estimating the higher heating value of biomass fuels from basic analysis data. *Biomass & Bioenergy, 28*, 499-507.

Triolo, J.M., Pedersen, L., Qu, H., Sommer, S.G., (2012). Biochemical methane potential and anaerobic biodegradability of non-herbaceous and herbaceous phytomass in biogas production. *Bioresource Technology, 125*, 226–232. https://doi.org/10.1016/j. biortech.2012.08.079.

Walker, D.J., Lutts, S. (2014). The tolerance of *Atriplex halimus* L. to environmental stresses. *Emirates Journal of Food and Agriculture*, *26*, 1081-1090.

Zhang, Y., Zhang, Z., Suzuki, K., Maekawa, T. (2003). Uptake and mass balance of trace materials for methane producing bacteria. *Biomass & Bioenergy, 25*, 427-33.

Zhao, Y., Xia, X., Yang, Z. (2013).Growth and nutrient accumulation of *Phragmites australis* in relation to water level variation and nutrient loading sin as hallow lake. *Journal of Environmental Science, 25*, 16-25.

Zimbardi, P., Cardinale, G., De Michele, M., Nanna F., Viggiano, D., Bonini, C., D’Alessio, L., D’Auria, M., Teghil, R., Tofani, D. (1999). *La lignina: una risorsa da valorizzare*. ENEA, Ente per le Nuove Tecnologie, L’Energia e L’Ambiente. https://www.osti.gov/etdeweb/servlets/purl/20078993 (accessed 2 December 2020).

6. SUMMARY AND CONCLUSIONS

6. SUMMARY AND CONCLUSIONS

In order to address the increasing demand for energy production and at the same time deal with environmental protection issues, the European Union has set the goal of increasing the share of renewable energy to 20%, among other objectives of the Europe 2020 Strategy. Some plant species have particular physical and chemical characteristics for their use as biomass crops for bioenergy production, with clear advantages both in terms of energy production and at the economic level. Moreover, it is clear that the different techniques used to obtain bioenergy require different characteristics in plant biomass. For example, the biomass for direct combustion should have at harvest both low water content (moisture < 10-12 %) and ash content, and a high concentration of lignin. For the production of bioethanol or for gasification, the plants may have high concentrations of sugars and lignocellulosic components (such as cellulose and hemicellulose) able to release sugars. The most suitable materials for the production of biogas through anaerobic digestion are those with a high concentration of biodegradable organic matter and low ash content.

The use of plant biomass for the production of bioenergy can therefore be considered a suitable and environmentally friendly option to achieve the desired renewable energy share. Of particular interest are a number of promising native wild plant species (non-food crops) such as those described in this Thesis (*A. donax, A. halimus, B. bituminosa, D. viscosa, F. vulgare, P australis, P. miliaceum* and *Z. fabago*), which can be very valid alternative raw materials for the production of (bio)energy. Furthermore, this could be done through innovative biocompatible and economically sustainable techniques that are currently being defined and / or developed, particularly in southern European countries. The low fertilization requirements and high adaptability to different soil types, including degraded and contaminated sites, of some of these species compared to conventional energy crops, together with their elevated energy yield potential, make the cultivation of these plants both economically and environmentally profitable. In addition, the competition for land use and agricultural resources with food crops would be avoided, this meaning a relevant step towards the necessary sustainability of agroecosystems in Southern Europe.

In particular, the experimental data considered in this study showed that the concentration of trace elements in the plants from mining sites did not affect their biogas production potential, or the production of thermal energy according to the high heating value (HHV) found for the studied species. In particular, the species *P. miliaceum*, *P. australis* and *A. donax* have shown the best perspectives for the production of biogas. Contrastingly, *D. viscosa* and *A. halimus* have adequate characteristics for their direct combustion, due to their low ash and moisture content and their high lignin concentrations, but less useful for biogas production by anaerobic digestion. Therefore, the most suitable species for the production of biogas seem to be those belonging to the Poaceae family, and the presence of high Mg and Na concentrations in the plants (aerial parts) conditioned the production of energy by anaerobic digestion more than the presence of potentially toxic trace elements (TEs) . Furthermore, the positive correlations between the values of HHV and biogas production suggest that the species investigated can be useful for phytoremediation of TEs contaminated soils, and their biomass used for the production of either thermal energy by combustion or biogas by anaerobic digestion.