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PRODUCCIÓN DE MICROALGAS EN DIFERENTES CONDICIONES DE CULTIVO Y SU EFECTO EN LA Y BIOSÍNTESIS DE NANOPARTÍCULAS DE COBRE

Tesis

Que presenta GERARDO SALAS HERRERA
como requisito parcial para obtener el Grado de
DOCTOR EN CIENCIAS EN AGRICULTURA PROTEGIDA

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PRODUCCIÓN DE MICROALGAS EN DIFERENTES CONDICIONES DE CULTIVO Y SU EFECTO EN LA Y BIOSÍNTESIS DE NANOPARTÍCULAS DE COBRE

Tesis

Elaborada por Gerardo Salas Herrera como requisito parcial para obtener el grado de Doctor en Ciencias en Agricultura Protegida con la supervisión y aprobación del Comité de Asesoría

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INTRODUCCIÓN

El termino microalgas, se refiere a microorganismos fotosintéticos con características morfológicas, fisiológicas y reproductivas muy variadas. Entre estas tenemos las diatomeas, algas verdes (Chlorophytas), algas rojas (Rodophytas), algas cafés (Phaeophytas) y cianobacterias que se desarrollan en una gran variedad de ambientes (Siezen, 2010). Estos microorganismos han despertado gran interés científico y biotecnológico en las últimas décadas para resolver problemas ambientales como bioremediación de aguas contaminadas (Dwivedi, 2012; Hii *et al.*, 2011; Siriam y Seenivasan 2012), así como para la producción de biocombustibles, productos farmacéuticos, bioreguladores agrícolas y la biosíntesis de nanopartículas metálicas.

Las condiciones en que se cultivan las microalgas impactan sobre el flujo del metabolismo celular como respuesta al ambiente en que se desarrollan. Estos cambios en el metabolismo pueden darse por respuesta a condiciones de estrés provocadas por disponibilidad de nutrientes, temperatura, iluminación, salinidad y fase de desarrollo del cultivo. Estas variaciones metabólicas en microalgas, ha sido ampliamente estudiada en la búsqueda de mejorar procesos biotecnológicos para la obtención de productos como lípidos, ácidos grasos poli-insaturados y carotenoides (Yu *et al*., 2011).

La producción de nanopartículas (NPs) metálicas mediante la biosíntesis a través de microalgas se ha explorado para el desarrollo de tecnologías ambientalmente amigables (Li *et al.* 2011), sin embargo, su producción depende fuertemente de las condiciones experimentales de los cultivos (Sudha *et al.*, 2013), área en la que aún falta mucho por investigar. La capacidad de las microalgas para producir nanopartículas metálicas está relacionada con los procesos de detoxificación por la presencia de metales pesados en el medio (Mohseniazar *et al*., 2011; Jena *et al*., 2014), respondiendo con el aumento en el contenido proteico así como un aumento significativo en la actividad de la catalasa, la superóxido dismutasa y en el contenido de glutatión reducido (Sabatini *et al*., 2009), además de la producción de metalotioninas y fitoquelatinas (Perales-Vela *et al*., 2006). También se ha encontrado evidencia de la relación que existe con proteínas oxido reductoras involucradas en la síntesis y transporte de ATP (Barwal *et al*., 2011). Estos compuestos además de moléculas antioxidantes no enzimáticas como pigmentos, polisacáridos y polifenoles también están relacionados con el estrés oxidativo que puede resultar de condiciones ambientales como la salinidad y la iluminación, así como por la presencia de metales o sustancias químicas (Cirulis *et al*., 2013), por lo que es de esperarse que las variaciones ambientales generen cambios metabólicos que hagan variar la capacidad para la producción de nanopartículas.

En este estudio se evaluaron los efectos de las condiciones de salinidad e iluminación en el cultivo de tres cepas de microalgas y en los efectos que estas condiciones tienen sobre la capacidad de estas cepas para la biosíntesis de nanopartículas de cobre. Además, tomando en cuenta que para el cultivo de microalgas se requieren grandes cantidades de agua y nutrientes, se hace una revisión de literatura de los factores que intervienen para la producción de microalgas usando como medio de cultivo aguas residuales urbanas en las diferentes etapas de tratamiento. Por otra parte, como complemento del presente trabajo, se añaden dos capítulos del libro "Agricultural Nanobiotechnology, modern agriculture for a sustainable future", en los cuales se participó como coautor.

REVISIÓN DE LITERATURA

Producción de microalgas

La producción de microalgas para distintos fines ha sido un tema que por muchos años ha capturado el interés científico por los múltiples aprovechamientos que se pueden tener de estos microorganismos. Para el consumo humano, se sabe del aprovechamiento de florecimientos naturales de *Nostoc* hace 2000 años en China (Spolaore *et al.*, 2006), así como el consumo de espirulina por los Aztecas Nicoletti (2016). Hoy en día, algunas microalgas son producidas como complemento alimenticio, así como para la producción de alimento en la industria acuícola Alkhamis y Qin (2013). La producción de biocombustibles usando microalgas, es otro tema en el que se han realizado muchas investigaciones, como ejemplo los esfuerzos realizados de 1978 a 1996 por el departamento de energía de Estados Unidos a través del programa de especies acuáticas en el Laboratorio Nacional de Energías Renovables Sheehan *et al.*, (1998). Algunos de estos esfuerzos y resultados más recientes sobre el tema fueron capturados por Gordon y Seckbach (2012) en el libro "The Science of Algal Fuels". Por otra parte, el aprovechamiento de aguas residuales para la producción de biomasa de microalgas para distintos fines, entre los que encontramos la producción de biocombustibles, ha sido un tema de investigación que ha inspirado muchos trabajos. Como ejemplo, los trabajos de Oswald y colaboradores desde 1957 sobre los sistemas de pilas de algas de alta tasa (High rate algal pond HRAPs), que se siguen desarrollando e investigando hasta nuestros días (Craggs *et al.*, 2012; Montemezzani *et al.*, 2017). Este cúmulo de investigaciones, aunado a otros temas relacionados con microalgas, ha generado un amplio conocimiento de estos microorganismos, así como un amplio desarrollo técnico científico para su aprovechamiento y producción.

Uno de los aspectos importantes a considerar en la producción de microalgas mediante sistemas autotróficos es la iluminación. La mejor fuente de luz por costo y disponibilidad es la natural. De la radiación que alcanza la superficie terrestre alrededor del 50% corresponde a la radiación fotosintéticamente activa (longitud de onda de 400 a 700 nm) y de esta la conversión efectiva a biomasa en un cultivo de microalgas corresponde aproximadamente a un 5% (Gordon y Seckbach 2012). La luz directa del sol es muy intensa para que sea completamente utilizada por las microalgas y el exceso de energía absorbida por las células es disipada en forma de fluorescencia o calor, la exposición prolongada a alta irradiancia puede sobrecargar los mecanismos de disipación de energía de las células resultando en fotoinhibición y daño celular (Christenson y Sims 2011). Sforza *et al.*, (2012), estudiaron los efectos de la luz en cultivos de *Nanochloropsis salina*, encontrando que entre 5 y 150 µM $m^{-2}s^{-1}$ aumentaba la tasa de crecimiento con el aumento en la iluminación, sin embargo, por arriba de los 150 µM $m^{-2}s^{-1}$ el aumento en la iluminación (350 y 1000 µM $m^{-2}s^{-1}$) presentaba disminución del crecimiento poblacional. Por otra parte, reportan que; a intensidades lumínicas de 1000 µM $m^{-2}s^{-1}$ la tasa de crecimiento fue similar a la obtenida a 350 µM $m^{-2}s^{-1}$, lo que indica que las células pueden mantener su sistema de protección y aun así continuar con el crecimiento poblacional. El uso de la luz solar para el cultivo de microalgas por lo general es usado para sistemas abiertos o fotobioreactores al aire libre. Las desventajas se relacionan con la variación en intensidad lumínica dependiendo de la hora del día, temporada del año y condiciones climáticas, así como la exposición a las variaciones ambientales.

En cultivos bajo techo con condiciones ambientales más controladas la iluminación más utilizada es la artificial (Ting *et al.*, 2017). Esto aumenta los costos de operación por el gasto en energía requerido en la iluminación. Para un fotobioreactor de 40 L se estima que se requieren de 40.32 kW/h con luz convencional a 20.16 kW/h con luz led, pudiéndose disminuir estos costos con el uso combinado de fibra óptica y la producción de energía eléctrica por celdas solares o energía eólica (Chen *et al.*, 2011). Otro aspecto importante a considerar en el uso de luz artificial es la calidad de esta, ya que diferentes fuentes de luz artificial ofrecen diferentes espectros dentro de radiación PAR, lo que puede afectar positiva o negativamente el crecimiento poblacional del cultivo de microalgas (Chang *et al.*, 2011). Por otra parte, el control de la calidad de la luz y el uso de luz monocromática (azul y roja) permite obtener diferentes respuestas metabólicas, Kim *et al.*, (2014) encontraron diferencias en la concentración y saturación de ácidos grasos con *Nannochlorpsis gaditana* al hacer variar la calidad de la luz en los cultivos. Con el uso de luz artificial, el fotoperiodo es fácilmente controlado y con esto sus efectos en el desarrollo del cultivo (Agrawal 2012; Alkhamis y Qin 2013). Por lo tanto, el control de la intensidad, calidad de la luz y fotoperiodo permiten la operación de fotobioreactores más eficientes para la producción de biomasa y metabolitos. Las desventajas que se tienen con la luz artificial son principalmente por el gasto energético y la inversión requerida en infraestructura.

Biosíntesis de nanopartículas metálicas

La biosíntesis de nanopartículas metálicas y de óxidos metálicos, ha cobrado gran interés en diferentes áreas de la ciencia en las últimas décadas. Se han utilizado diferentes tipos de extractos naturales para su biofabricación como biocomponentes extraídos de hongos, bacterias, levaduras, algas y plantas. Entre los procesos en que estas NPs se pueden formar encontramos aquellos en los que se utilizan extractos de diferentes órganos de la planta (hojas, flores, frutos, semillas) o la planta completa y los procesos en los que intervienen procesos metabólicos de los organismos vivos (Singh *et al.*, 2018). Se ha comprobado que los extractos orgánicos poseen agentes de alta eficiencia para la reducción y estabilización de NPs metálicas. De forma general la reducción de los iones metálicos y la estabilidad de las NPs es atribuida a compuestos orgánicos presentes en los extractos, algunos de los compuestos que podrían estar involucrados en la reducción y estabilización de estas partículas son: fenoles, esteroides, proteínas, azucares reductores, entre otros. Las plantas contienen biomoléculas como carbohidratos, proteínas y coenzimas, los cuales varían su concentración dependiendo de la planta que se trate, el metabolismo de esta y el órgano utilizado para la elaboración del extracto.

Biosíntesis de nanopartículas metálicas con microalgas

Las microalgas son fuente de biomoléculas que pueden participar en la reducción de sales metálicas para la formación de NPs metálicas. Estas mismas moléculas además pueden participar en la protección de las NPs formadas. En su revisión Siddiqi y Husen (2016), comentan que entre los agentes reductores y estabilizadores aportados por las algas encontramos proteínas, polisacáridos, aminas, aminoácidos, alcoholes, pigmentos, ácidos carboxílicos, carbohidratos y azucares. Estos mismos autores destacan el alto potencial de las microalgas para la biosíntesis de NPs metálicas de una manera amigable con el ambiente. Esto, al evitarse la utilización de productos químicos tóxicos y la alta demanda energética requerida para la síntesis fisicoquímica.

En la biosíntesis de NPs con microalgas pueden intervenir metabolitos celulares liberados al medio de cultivo sin la presencia de células o mediante la intervención de células vivas, siendo esto comprobado para la biosíntesis de Ag NPs por Barwal *et al.,* (2011) utilizando a *Chlamydomonas reinhardtii* como organismo modelo y por Patel *et al.,* (2015), quienes evaluaron ocho cepas de microalgas eucariotas y 8 cepas de cianobacterias. En su trabajo Barwal *et al.,* (2011), reportan la intervención de proteínas oxido reductoras y la intervención de la maquinaria molecular en la formación de las NPs. Por otra parte, Patel *et al.,* (2015), comentan la intervención de polisacáridos celulares y C-ficocianinas en el caso de cianobacterias. Concluyendo que es necesaria la presencia de una molécula orgánica y la intervención de la luz para la formación de las NPs de plata.

Los efectos de las condiciones de cultivo en el que se desarrollan las microalgas pueden afectar la calidad en cuanto a forma y tamaño de las NPs síntetizadas. Soleimani *et al*., (2017) encontraron el pH de la suspensión de algas afecta la forma de las NPs de plata sintetizadas reportando que con pH bajo o neutro se obtienen formas esféricas mono dispersas, mientras que con pH alcalino se formaron nanobarras. Otro factor que puede afectar la forma de las NPs es la relación entre el agente reductor (extractos, metabolitos en el medio de cultivo o número de células) y la sal metálica utilizada (Dahoumane *et al*., 2017).

La posibilidad de controlar la formación, tamaño y estabilidad de NPs metálicas mediante procesos ambientalmente amigables y con buena relación costo-beneficio, hacen que sea atractiva la biotecnología con microalgas para este fin. El control de los parámetros de cultivo como son iluminación, salinidad, pH, temperatura y nutrientes, pueden afectar significativamente la actividad enzimática de estos microorganismos (Shah *et al.,* 2015), por consecuencia hacer variar los posibles resultados en la formación de NPs metálicas.

Posibles aplicaciones de nanopartículas en la agricultura

Hay un amplio campo de aplicaciones y desarrollo en el área de la nanotecnología en muchas áreas del conocimiento. Sin embargo, en la agricultura su desarrollo aún es marginal. Según Parisi *et al.,* (2015), esto se debe a los altos costos de producción de estos productos, los cuales se requieren en grandes volúmenes en el sector agrícola, además de que los beneficios técnicos aún no han sido claros, hay falta de certeza legislativa y hay incertidumbre de la opinión pública. A pesar de esto, los autores comentan que las investigaciones y el desarrollo de la nanotecnología son muy prometedora y se exploran muchas aplicaciones de esta en la agricultura. Más recientemente, Worrall *et al*., (2018), revisaron el uso de la nanotecnología para el manejo de enfermedades de las plantas. Donde concluyen que se requiere del trabajo multidisciplinario entre expertos en el estudio de materiales y de la biología para tener un entendimiento profundo de los mecanismos de interacción en un sistema biológico a nivel nanométrico. Un entendimiento comprensivo a cerca de las propiedades de las NPs, tales como: morfología, tamaño, grupos funcionales, y capacidad activa de adsorción y carga, podrían proveer una guía útil como punto de arranque para la selección racional de las nanopartículas adecuadas. Las soluciones a problemas de plagas agrícolas a través de la nanotecnología la resumen en siete potenciales ventajas:

1) Solubilidad mejorada de pesticidas de baja solubilidad,
2) Aumento de la biodisponibilidad y eficiencia de pesticidas,
3) Aumento de la vida de anaquel y la deliberación controlada de los componentes activos.
4) Liberación de las moléculas activas a blancos específicos y dependiendo del pH.
5) Liberación de moléculas de RNAi para el manejo de plagas.
6) NPs como transportadores de moléculas activas mejorando la estabilidad UV de las formulaciones.
7) Nanopesticidas para mejorar la toxicidad selectiva y superar los problemas de resistencia a pesticidas.

Estas ventajas se enfocan en la protección de las plantas de enfermedades y principalmente como vehículo de los compuestos activos, sin embargo, hay otros campos de aplicación de estas tecnologías como: detección de patógenos, detección de residuos de pesticidas, acelerar la germinación y producción (Khot *et al.,* 2012), aumentar la producción y el uso eficiente del agua y fertilizantes (Sekhon BS 2014). Por otra parte, Duhan *et al.,* (2017), hacen referencia a la nanotecnología como la nueva perspectiva en la agricultura de precisión. Esto destacando la deliberación de materiales a través de la mediación de las NPs y biosensores avanzados para la producción de precisión, los cuales son solo posibles por nanopartículas o nanochips. La nanoencapsulación de fertilizantes convencionales, pesticidas y herbicidas ayudan a tener una liberación lenta y sostenible de nutrientes y agroquímicos, resultando en una dosis precisa en las plantas. Otro aspecto que comentan los autores es la detección temprana de enfermedades virales basada en kits nanotecnológicos para este fin.

A pesar de estas aplicaciones benéficas de los nanomateriales para la agricultura, aún hay incertidumbre sobre la seguridad de estos en cuanto a su impacto al medio ambiente a largo plazo y su impacto a otras entidades biológicas. Las aplicaciones prácticas de la nanotecnología permanecen bajo la incertidumbre, esto debido a la baja capacidad de controlar las propiedades a escalas nanométricas, al efecto ambiental y a la falta de una base de datos de toxicidades (He *et al.,* 2019). Por lo anterior, Kaphle *et al.,* (2018) recomiendan el diseño racional de los nanomateriales para proveer oportunidades adicionales previniendo posibles efectos hostiles. Algunos autores sugieren el establecimiento de modelos que representen lo mejor posible los parámetros que pudieran afectar la toxicidad de estos materiales, para tener una idea de la posible toxicidad antes de su aplicación al medio ambiente (Dasgupta *et al.,* 2017; Kaphle *et al.,* 2018). Por otra parte, Nayantara y Kaur (2018), concluyen que se podrían utilizar nanopartículas biosintetizadas para el control de fitopatógenos, las cuales representarían una menor toxicidad para los humanos que la de algunos pesticidas sintéticos comercialmente disponibles.

La fitotoxicidad de NPs de metales esenciales fue revisada recientemente por Ruttkay-Nedecky *et al.,* 2017, quienes entre sus conclusiones destacan que las NPs de metales esenciales y sus óxidos han probado ser adecuados para su uso en la agricultura. Entre estos, los óxidos de fierro y manganeso podrían ser los menos fitotóxicos. Por otra parte, Taran, *et al.,* (2014), evaluaron la redistribución de elementos metálicos (Fe, Cu, Mn y Zn) en el tejido de plantas de trigo bajo tratamiento de NPs metálicas biosíntetizadas no iónicas en solución coloidal. Encontraron que las NPs tienen la habilidad de penetrar la cobertura de las semillas y el efecto de la aplicación es dependiente de la composición de las NPs en solución y la forma de aplicación. Además, los autores comentan que en aplicaciones a partes aéreas de los nanomateriales, la penetración se da vía epidermis y estomas y son transportadas rápidamente dentro de la planta e incluidas en los procesos metabólicos. Comentan que las fluctuaciones detectadas en el contenido de elementos metálicos en los tejidos de las plantas, podría relacionarse con la regulación a nivel celular y la optimización de procesos metabólicos donde los metales son requeridos, sin detectar la acumulación de metales en los tejidos de las plantas evaluadas. La utilización de NPs metálicas en estudios recientes también fu reportado por Chaudhuri y Malodia (2017) en vivero de árboles, encontrando un crecimiento más rápido en plántulas tratadas con NPs de zinc sintetizadas a partir de extractos de hojas.

Se ha demostrado la actividad antibacterial de diferentes NPs de metales sintetizadas a partir de extractos vegetales contra distintas bacterias patógenas para el ser humano, por ejemplo con NPs de óxido de zinc contra *Escherichia coli* y *Pseudomonas aeruginosa*, (Shamila *et al.,* 2018), está última con capacidad de infectar también a plantas, NPs de plata contra *Escherichia coli* y *Staphylococcus aureus* (Behravan *et al.,* 2019) y otros agentes patógenos (Larayetan *et al.,* 2019; Wintachai *et al.,* 2019; Maanvizhi *et al.,* 2018). El tratamiento de enfermedades en las plantas con el uso de NPs metálicas es otro tema que ha cobrado gran interés para su aplicación en la agricultura. Se han encontrado efectos fungicidas y fungistáticos de NPs de cobre protegidas con quitosano aplicadas a cultivos en placa de *Rhizotocnia solani* y *Sclerotium rolfsii* (Rubina *et al.,* 2017). Con formulaciones acuosas de nano sulfato de cobre NCuS, Sidhu *et al.,* (2017), encontraron que la formulación es 10 veces más potente que lo obtenido con el fungicida "captan" después de tratar las semillas de arroz con la nano-formulación, las evaluaciones antifúngicas las realizaron con inóculos de *Alternaria alternata, Drechslera oryzae* y *Curvularia lunata,* encontrando además un efecto favorable en la germinación y parámetros de crecimiento. En su estudio reportan el tratamiento de las semillas con una concentración de 5-10µg mL^{-1} de NCuS. Por otra parte, Husain *et al.,* (2017), reportan efectos negativos en la germinación y provocación de estrés en semillas de *Artemisia absinthium* tratadas con nanopartículas de cobre en una concentración aproximada de 30 µg mL^{-1}, teniendo efecto prolongado en la germinación, perfil bioquímico y crecimiento de las plantas.

El efecto de tratamiento de semillas en pre siembra con NPs de metales en la formación de reacciones de defensa de plántulas de trigo infectadas con el agente causal de la Cercosporelosis, fue evaluada por Panyuta *et al.,* (2016). Los autores comentan que las NPs metálicas evaluadas (Zn, Ag, Fe, Mn, Cu) pueden aumentar las propiedades antioxidantes de las células bajo condiciones de estrés causadas por el patógeno. Pudiendo estar relacionado con un aumento en las reacciones de defensa endógenas contra el patógeno. Aunado a esto, los autores comentan una menor inducción de lectina en tratamientos con NPs de cobre, posiblemente relacionado con las propiedades fungicidas del cobre. Este efecto indirecto de aumento en los mecanismos de resistencia en plántulas de trigo también fue evaluado por Taran N *et al.*, (2017), solo que en cuanto a la resistencia a la sequía en dos variedades. Los autores reportan que las NPs evaluadas (Cu-Zn NPs) tuvieron un efecto positivo en el balance pro-oxidativo / anti-oxidativo, principalmente en uno de los eco tipos, lo anterior le dio una mayor resistencia a las condiciones de sequía.

En conclusión, hay un amplio campo de aplicaciones benéficas de NPs metálicas para la agricultura. Las formas no-iónicas de NPs de metales esenciales, posiblemente se vean en el mercado en algunos años. Algunas de las aplicaciones donde podríamos verlas es como micronutrientes con aplicaciones más eficientes por lo tanto menor volumen de los compuestos, como agentes fungicidas y como inductores de mecanismos de resistencia a patógenos y/o condiciones adversas del clima. Sin embargo, aún hay un amplio campo de investigación. Por una parte, en la síntesis, donde el uso de microorganismos y extractos vegetales tiene un gran potencial. Por otra parte, la identificación de las mejores presentaciones en cuanto a tamaño, morfología, dispersión, agentes protectores de la nanopartícula, estabilidad y mecanismos de acción.

PRIMER ARTÍCULO

Impact of microalgae culture conditions over the capacity of copper nanoparticles biosynthesis

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Impact of microalgae culture conditions over the capacity of copper nanoparticle biosynthesis

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Abstract

The biosynthesis of metallic nanoparticles (NPs) has been previously reported using a variety of organic molecules produced by microalgae. However, the results obtained could vary due to the metabolic responses that microalgae have to different culture conditions which could affect the characteristics of the produced nanoparticles. In the present report, copper nanoparticle formation was evaluated by the microalgae *Chlorella kessleri*, *Dunaliella tertiolecta*, and *Tetraselmis suecica*, developed under combined conditions of low (L−) and high (L+) illumination, with low (S−) and high salinity (S+). The illumination was 12 h:12 h light/dark. NP formation was evaluated 72 h after exposure to copper salt. Cupric oxide (CuO) NPs were detected spectrophotometrically in both the culture media (extracellular NPs) and cells (intracellular NPs) of *Ch. kessleri* with absorbance in the range of 200 to 235 nm. Metallic copper NPs (Cu_n) were detected with an absorbance between 540 and 560 nm in treatments with cells of *C. kessleri* and *D. tertiolecta* which were grown in *L+S−*, while *T. suecica* cells showed Cu_n NPs formations in *L−S−*, *L−S+*, and *L+S−*. The size difference of the NPs was measured by scanning electron microscopy (SEM), in treatments with cells of *C. kessleri*, ranging in size from 15 to 25 nm (*L−S−*) and 55 to 65 nm (*L+S−*). In treatments with culture media, sizes from 35 to 45 nm (*L−S−*) of NPs were obtained. Differences in the biosynthesis of Cu-based NPs are possible, depending on the culture conditions and the strain of microalgae to be utilized.

Keywords Phyconanotechnology · *Chlorella kessleri* · *Dunaliella tertiolecta* · *Tetraselmis suecica* · Salt stress · Cupric oxide

Introduction

The production of inorganic NPs through biosynthesis by microorganisms such as bacteria, fungi, and microalgae has been explored by several authors for the development of low-cost and eco-friendly technologies (Li et al. 2011). Microalgae have been highlighted as a potential source of diverse biomolecules such as proteins, pigments, carbohydrates, alkaloids, terpenes, peptides, and some aromatic compounds, which may be involved in the reduction of metal ions for the formation and stabilization of metal NPs, without producing toxic by-products (Siddiqi and Husen 2016). Copper-based NPs are of great industrial interest since they have similar properties to other NPs which are based on less abundant metals (Shobha et al. 2014). These NPs' properties allow their application in several processes, such as in catalysis (Gawande et al. 2016), gas sensors, solar energy transformation, and semiconductors (Singh et al. 2016). In agriculture, copper NPs have been used to control several pathogens such as fungi and some bacteria (Singh Sekhon 2014), as well as being used as a crop production enhancer (Hafeez et al. 2015). However, Shobha et al. (2014) report that only 5% of the research papers in the area of nanoparticle biosynthesis correspond to copper-oxide (CuO) NPs, with the major focus being on the production of silver NPs, accounting for 59% of the publications.

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The biosynthesis of compounds at the nanometer scale could be by way of either biochemical or biological processes (Morales-Díaz et al. 2016). Biochemical processes take advantage of biomolecules derived from algae extracts (Abboud et al. 2013) or by metabolites secreted to the culture media (Patel et al. 2015), while biological processes take advantage of live cells for intracellular biosynthesis (Jena et al. 2014), or are related to metabolic process such as the electron transport chain (Shabnam and Pardha-Saradhi 2013).

The capacity of microalgae to produce metallic NPs has been related with detoxification processes, due to the presence of heavy metals in the environment where cell development occurs (Mohseniazar et al. 2011). In this mechanism, microalgal cells respond by increasing the protein content, as well as catalase activity, superoxide dismutase and the content of reduced glutathione (Sabatini et al. 2009), and phytochelatin production (Miazek et al. 2015). Also, evidence has been found on the oxide-reductive protein relationship involved in the synthesis and transport of adenosine triphosphate (Barwal et al. 2011). Those compounds, as well as non-enzymatic antioxidant molecules, such as pigments, polysaccharides, and polyphenols, are related to oxidative stress which could be a result of the negative environmental conditions and the presence of adverse metals or chemical substances (Cirulis et al. 2013). Among the environmental conditions that may produce an increase in oxidative stress in microalgae and promote the production of antioxidant compounds are salinity and lighting. As a response to salinity stress, microalgae vary their secretion of biopolymers (exopolysaccharides) and phyto-hormones (abscisic acid and indoleacetic acid) to the media (Liu et al. 2016). Secreted polysaccharides could be sulfated and are conformed mainly for glucose, xylose, and galactose with different proportions (Raposo et al. 2013). On the other hand, light stress induces acclimation by optimizing the cell photosynthetic apparatus and increasing the antioxidant defense mechanisms (Simionato et al. 2011). Microalgal metabolite production strongly depends on the culture conditions, such as temperature, pH, incubation time, culture media composition, and light intensity (Sudha et al. 2013). These factors could be manipulated to influence the size and morphology of the microalgae synthesized NPs (Li et al. 2011; Dahoumane et al. 2017). Considering the scarcity of information on the biosynthesis of Cu-NPs by microalgae and the possible effects that the culture conditions could have on their production, the objective of this study was to evaluate the culture condition effect on the obtaining of Cu-NPs, and its influence on size through biosynthesis with microalgae under different conditions of illumination and salinity.

Materials and methods

Microalgae strains and experimental conditions

Microalgae strains of *Dunaliella tertiolecta* and *Tetraselmis suecica* were provided by the company Biorganix Mexicana from their private collection. A strain of *Chlorella kessleri* (CDBB-A-12) was acquired at "Colección nacional de cepas microbianas y cultivos celulares" from the "Centro de Investigaciones y de Estudios Avanzados del Instituto Politécnico Nacional." Strains were cultivated in f/2 medium for salt water strains (Guillard and Ryther 1962) and Bold medium (Jena et al. 2014) for *C. kessleri*. All material was sterilized in an autoclave before use. All culture inoculations were done in a laminar flux chamber under aseptic conditions. An Erlenmeyer flask of 250 mL with 150 mL of culture media was inoculated with 3×10^5 cells mL^{-1}. All experimental units were kept with constant aeration, illuminated at the base of the flasks with a 17-W white fluorescent lamp (Phillips ALTO II T8) and incubated at 23 °C. A transparent polycarbonate sheet was used as a base for the experimental units. The lighting period was 12 h alternating with 12 h of dark. The process was monitored for 18 days after inoculation (0, 3, 5, 7, 9, 1, 13, 15, and 18 days), and 1 mL of sample was taken from each experimental unit and analyzed immediately. Photosynthetically active radiation (PAR) was measured with a quantum sensor (LightScout 3681) over the polycarbonate sheet. The measured PAR radiation reported corresponds to that received at the flask base. For the treatments, PAR factors of low radiation (−) (50 μmol photons m^{-2} s^{-1}) and high radiation (+) (230 μmol photons m^{-2} s^{-1}) were combined with low and high salinity conditions as shown in Table 1. For the experiments, four treatments per strain were obtained with three replicates for each one.

Analytical methods

For each sample taken from the experimental unit at the days established, cell counting, pH (Horiba, Laqua twin S010), and electrical conductivity (Horiba Laqua twin S070) were monitored. Cell counting was done with a Neubauer chamber three times per experimental unit at the days evaluated. The growth rate (GR) was calculated with Eq. (1):

$$GR = (\ln x_2 - \ln x_1)/(t_2 - t_1) \quad (1)$$

where x_1 and x_2 represent the cell concentration at the time on days t_1 and t_2 (García et al. 2007), respectively.

To quantify the cell volume, the general method for calculating the cell biovolume based on geometric assignation was used. For *C. kessleri*, the sphere formula was employed, while for *D. tertiolecta* and *T. suecica*, this was calculated with the

Table 1 Experimental matrix with combinations of illumination and salinity that conform to the treatments for each strain. *PAR* photosynthetically active radiation, *EC* electrical conductivity

Strain	PAR (μmol photons m^{-2} s^{-1})	Salinity NaCl (M)	EC (mS cm^{-1})	pH
Ch. kessleri	50	0.00043	0.82	7.1
	50	0.10000	9.87	6.8
	230	0.00043	0.85	7.1
	230	0.10000	10.00	6.8
D. tertiolecta	50	0.46	55.3	7.7
	50	1.50	135.0	7.4
	230	0.46	55.0	7.7
	230	1.50	136.0	7.4
T. suecica	50	0.46	60.7	7.7
	50	1.00	105.0	7.5
	230	0.46	63.3	7.7
	230	1.00	105.0	7.5

prolate spheroid formula (Sun and Liu 2003). With pictures taken by optic microscope using the program Axion Vision 4.8, 30 cells were measured for each treatment, and the equatorial diameter of each cell was taken. In the cases of *D. tertiolecta* and *T. suecica* in addition to the diameter, length measurements were taken. The average volume of the cells in each treatment and the population density was used to calculate the biovolume of culture per milliliter.

For the photosynthetic pigment quantification, 2 mL of the culture medium was taken as mentioned above. Samples were centrifuged at 8000 rpm for 15 min, and precipitates were recovered. The pellet was then mixed with 1 mL of ethanol and sonicated (Bransonic 1510R-DTH) for 15 min. After 24 h in refrigeration, absorbances were measured at 470, 653, and 666 nm (Lichtenthaler and Wellburn 1983; Henriques et al. 2007).

Biosynthesis and evaluation of copper nanoparticles

After 18 days of culturing, the populations were counted to calculate the copper sulfate ($CuSO_4 \cdot 5H_2O$ reactive grade, Fermont) doses for each treatment. The maximum population of each strain was taken as a basis with a concentration of 0.5 mM of $CuSO_4$. Copper sulfate concentration was adjusted proportionally to each treatment according to its cell population, to assure the same concentration of copper sulfate per cell. Subsequent to the 72 h of copper sulfate application (21 days after inoculation), each experimental unit was divided into four flasks with 20 mL of sample for each one. Two flasks were kept as the original experimental unit (cells in their culture medium), and the other two flasks were cell-free, containing only the culture medium. The cell-free media flasks were obtained by centrifugation (Hermle Z 206 A) at 6000 rpm for 15 min and by separating the supernatant.

After 72 h of copper exposure, UV-vis spectroscopy evaluations were used (Thermo Scientific, Genesys 10S) to detect Cu-NP formation. The scanned region from 200 to 700 nm showed the characteristic absorbance peaks for Cu-NP identification. The surface plasmon resonance of the surfaces of Cu-NPs with spherical forms is localized in the visible region with a maximum absorbance between 520 and 580 nm (Pestryakov et al. 2004; Gawande et al. 2016), while the detection of different types of CuO-NPs (Table 2) was in the UV region of the spectrum (Pestryakov et al. 2004; Rahman et al. 2009; Bouazizi et al. 2015; Valli and Suganya 2015). Samples were kept in ultra-freezing conditions (− 80 °C) for later analysis by SEM (Hitachi SU8010) at a 3.0 kV accelerating voltage.

Statistical analysis

All experiments were set up with a randomized complete blocks design. Statistical analysis was done with the "R" program (Team 2016) utilizing the Duncan test ($\alpha \leq 0.05$) for mean comparison with the package "agricolae" (Mendiburu 2016). The effects of illumination and salinity over the final population and biovolume were analyzed by a two-way ANOVA factorial analysis (2 × 2). When the data to be analyzed did not meet the assumption of normality, these were transformed with the "car" package for R (Fox and Weisberg 2011).

Results

The strain with the best growth rate was *C. kessleri* (Table 3), with the highest values in the period from 0 to 9 days, showing a slight deceleration in the period from 9 to 18 days. The condition in which the growth rate was most affected for this strain was L−S+. Both irradiance and salinity showed a

Table 2 UV-vis spectrum regions where copper and copper oxide nanoparticles were reported

Nanoparticle type	UV-vis regions	Reference
Cu+	250 nm	(Pestryakov et al. 2004)
CuO	310 nm	(Rahman et al. 2009)
CuO *crystals*	650 nm	(Abboud et al. 2013)
	205–221 nm	(Bouazizi et al. 2015)
O-Cu-O (CuO_2)	320–370 nm	(Pestryakov et al. 2004)
Cu-O-Cu (Cu_2O)	260 nm	(Abboud et al. 2013)
	400–440 nm	(Pestryakov et al. 2004)
	542 nm	(Rahman et al. 2009)
Cu_n	520–580 nm	(Pestryakov et al. 2004)
	552–561 nm	(Dang et al. 2011)
	558 nm	(Suresh et al. 2016)
d-d transitions	620–850 nm	(Pestryakov et al. 2004)

Table 3 Microalgae growth rate (day^{-1}) means (± SD) obtained in each treatment ($n = 3$) at different time intervals. Means followed by the same letter are not significantly different at the 5% confidence level according to the Duncan multiple comparison test. Data were transformed with the "car" program for "R" to meet the assumption of normality

		Growth rate (day^{-1})		
Strain	Treatment	0 to 9 days	9 to 18 days	0 to 18 days
C. kessleri	L−S−	0.48 (0.027) ab	0.21 (0.017) a	0.70 (0.018) a
	L−S+	0.39 (0.027) cd	0.18 (0.033) ab	0.57 (0.043) bc
	L+S−	0.59 (0.047) ab	0.25 (0.011) a	0.75 (0.056) a
	L+S+	0.51 (0.062) ab	0.19 (0.041) ab	0.70 (0.040) a
D. tertiolecta	L−S−	0.51 (0.014) ab	0.06 (0.028) bc	0.58 (0.015) b
	L−S+	0.43 (0.024) bc	0.06 (0.031) bc	0.50 (0.028) efg
	L+S−	0.52 (0.001) a	0.02 (0.014) c	0.54 (0.013) bcd
	L+S+	0.49 (0.012) ab	0.03 (0.017) c	0.53 (0.005) cde
T. suecica	L−S−	0.28 (0.146) de	0.20 (0.18) ab	0.49 (0.033) g
	L−S+	0.21 (0.143) e	0.31 (0.15) a	0.52 (0.016) ef
	L+S−	0.26 (0.087) e	0.23 (0.082) a	0.50 (0.004) efg
	L+S+	0.31 (0.058) de	0.17 (0.059) ab	0.49 (0.023) fg

significant effect on the final population of this strain without interaction between factors (Fig. 1; Table 4). Lower populations and bigger cells of *C. kessleri* can be obtained with high salinity conditions. In the case of *D. tertiolecta*, the highest growth rate was found in the period of 0 to 9 days; however, a growth deceleration was present in the period of 9 to 18 days, reaching the stationary phase in all treatments (Fig. 2). The illumination effects were not significant for both the final population and cellular volume of *D. tertiolecta*; nevertheless, these factors interact with the salinity and have significant effects on the population and cellular volume (Fig. 1). The photosynthetic pigment content showed variations between treatments (Fig. 3), with the highest values in chlorophyll a L−S+ treatments, which could be related to adjustments in the photosynthetic apparatus to low illumination and salinity stress, reflected in the variations of carotenoids. On the other hand, *T. suecica* did not present significant effects or interactions due to illumination and salinity factors on the final

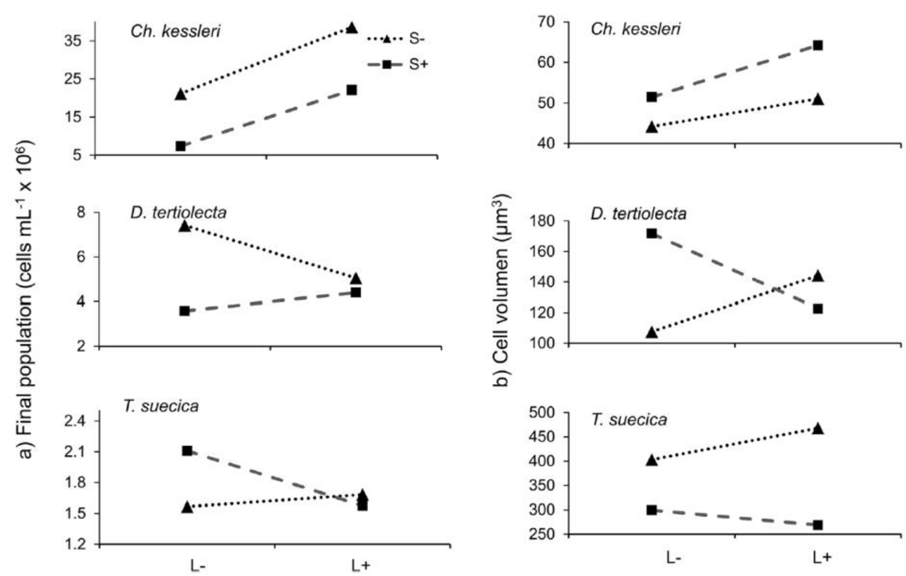

Fig. 1 Graphs of the illumination and salinity interaction on (a) the final population and (b) the cell volume at 18 days of culturing. Low salinity is represented by dotted lines and high salinity by hyphens. High or low illumination is indicated below the set of graphs

Table 4 Significance levels (*P* values) obtained from two-way ANOVA analyses for the effects of light and salinity and their interaction on (a) the final population ($n = 3$) and (b) cell volume ($n = 30$)

(a) Final population				(b) Cell volume			
	C. kessleri	*D. tertiolecta*	*T. suecica*		*C. kessleri*	*D. tertiolecta*	*T. suecica*
Illumination	0.026	0.116	0.305	Illumination	0.004	0.421	0.070
Salinity	0.034	0.001	0.287	Salinity	0.144	< 0.001	< 0.001
Interaction	0.827	0.0073	0.125	Interaction	0.277	< 0.001	< 0.001

population. The significant effects of those factors were only observed in the cell volume of this strain (Fig. 1), with the biggest cells in the treatments with low salinity. *Tetraselmis suecica* cultures in L+S− and L−S− treatments reached a stationary growth phase around 15 days of culturing (Fig. 2), while in L−S+ and L+S+ conditions, the population growth was observed until 18 days.

Comparing the maximum population (Fig. 4) and cellular volume evaluated for the three strains, *C. kessleri* was found to have the smallest cells (45 to 65 μm^3) and the highest populations, followed by *D. tertiolecta* with bigger cells (110 to 170 μm^3). The lowest populations were obtained with *T. suecica*, showing the biggest cells (270 to 470 μm^3). With the cell size data, the biovolume was obtained in each treatment, as well as the $CuSO_4$ concentration per cubic millimeter

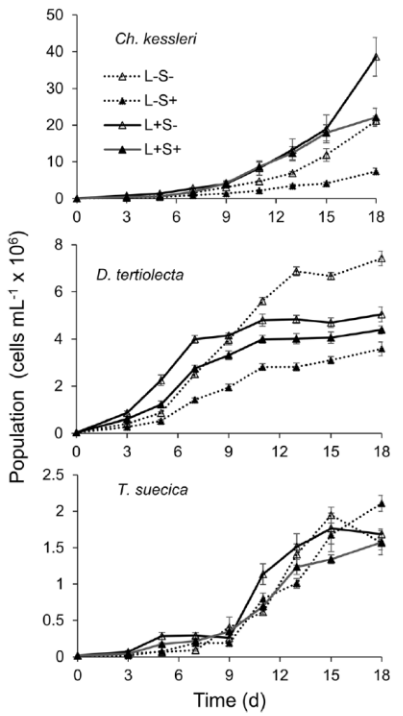

Fig. 2 Population growth curves. Dotted lines: low illumination, continuous line: high illumination, empty triangles: low illumination, solid triangles: high salinity. Data represents the average ($n = 3$), and standard error bars are shown

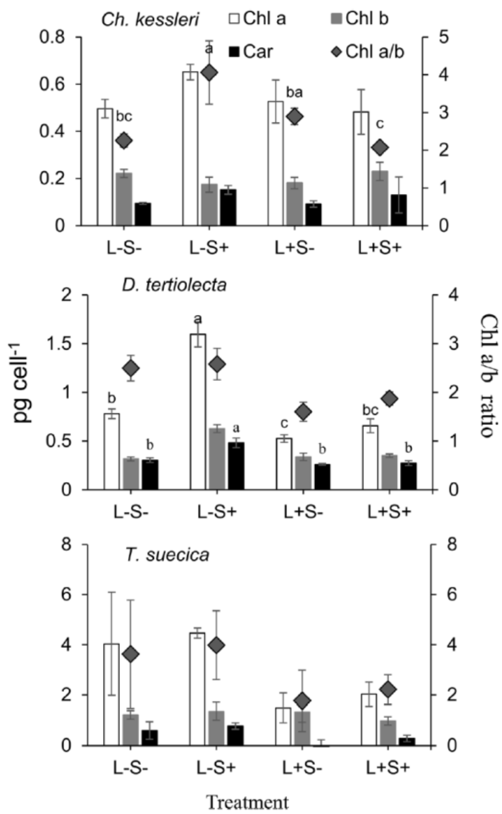

Fig. 3 Photosynthetic pigments and chlorophyll a/b ratio, with the *y* scale to the right of each graph. Means ($n = 3$) with different letters represent significant differences at the 5% confidence level (Duncan multiple comparison test). Standard error bars are shown. L light, S salinity, (−) low, (+) high

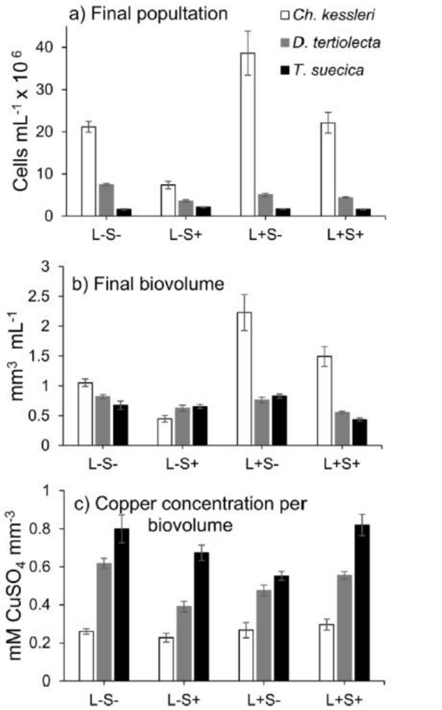

Fig. 4 Comparison between the distinct treatments and the three strains on **a**) final population, **b**) final biovolume, and **c**) mM $CuSO_4$ mm^{-3} biovolume mL^{-1}. White bars: *C. kessleri*, gray: *D. tertiolecta*, black: *T. suecica*. L light, S salinity, (−) low, (+) high. Data represents the average ($n = 3$), and standard error bars are shown

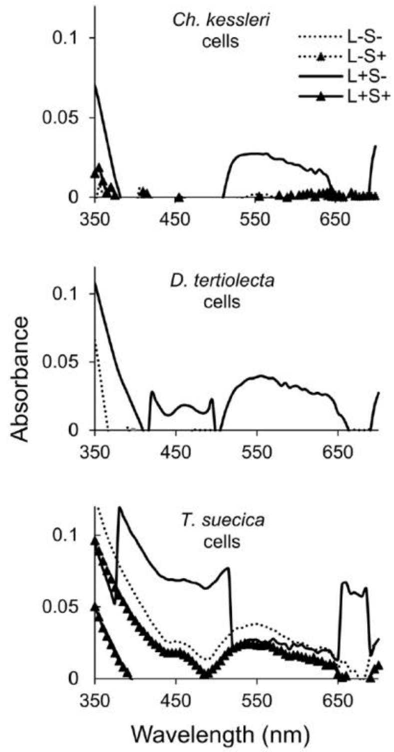

Fig. 5 Absorbances detected between 350 and 700 nm in treatments with cells 72 h after exposure to copper sulfate

of biovolume (Fig. 4b, c). This represents the relation obtained between the copper salt and the possible biological agents (biochemical or metabolic) involved in the NP biosynthesis.

Five of the 12 treatments with cells showed absorbance by UV-vis spectroscopy in the visible region, corresponding to Cu_n NPs. The culture condition which favored the formation of Cu_n NPs in the three strains was L+S−, in addition for *T. suecica* cells in the treatments L−S− and L−S+ (Fig. 5). High illumination has an important effect in copper ion reduction, mainly for *C. kessleri* and *D. tertiolecta*. The signs of Cu_n NP formation were found only in cell treatments and not with the culture media, if cellular metabolites were involved in their formation, not those secreted to the culture media. The copper concentration caused toxicity for *D. tertiolecta* and *T. suecica* in the treatments where Cu_n NP formation was detected and reflected in the culture bleaching; because of this, it is not clear if the copper ion reduction was by an intracellular mechanism linked to a metabolic process or by some reducing agent, which could be cellular biomolecules. On the other hand, *C. kessleri* showed higher tolerance to the copper concentration used; despite this, Cu_n formation was detected only under high illumination and low salinity.

The absorbances detected by UV-vis spectrometry in the UV region (Fig. 6) present a maximal wavelength of 200 to 280 nm, varying in intensity and amplitude. The formation of CuO NPs was detected with *C. kessleri* in treatments with cells in L−S− and L−S+ conditions and so the illumination played a more important role than salinity for the biosynthesis of CuO NPs with this strain. In contrast, with *D. tertiolecta* cells, CuO NPs were detected in both L−S− and L+S− conditions and so the high salinity could be a negative factor for the NP biosynthesis with this strain. In the treatment L+S− with cells of *C. kessleri*, NPs of between 55 and 65 nm were obtained (Fig. 7), which was the only treatment with this strain which registered absorbances of Cu_n (λ 550 nm) as well as CuO (λ 200 and 215 nm) nanoparticles. On the other hand, in the treatment L−S−, cells of *C. kessleri* NPs of between 15 and 25 nm were found with absorbances corresponding to CuO (205 nm). With the media in the treatment L−S− where *C. kessleri* was cultured, the nanoparticles found ranged from

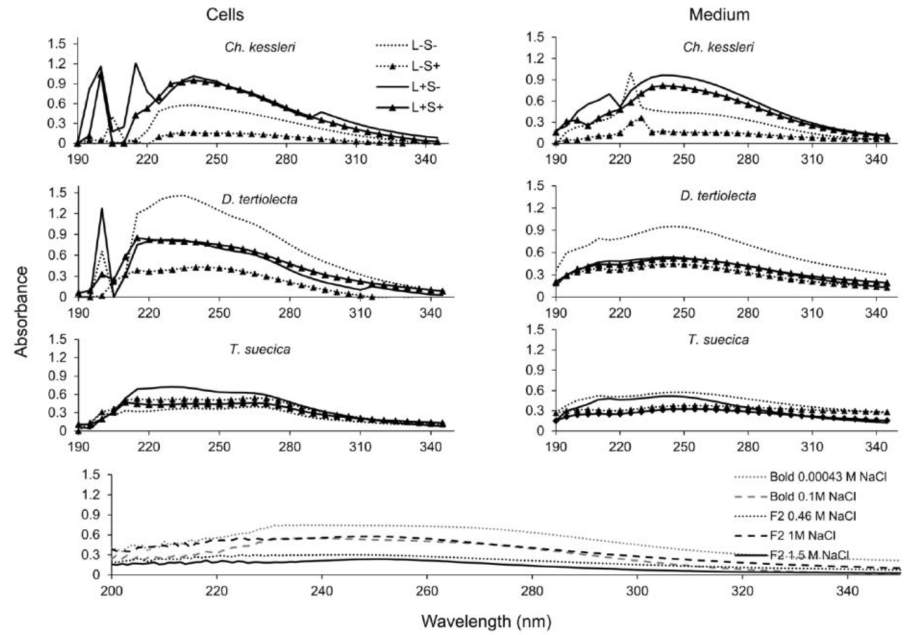

Fig. 6 Absorbances detected from 190 to 350 nm for the detection of copper nanoparticles. Dotted lines: low illumination, continuous lines: high illumination, lines without figures: low salinity, lines with triangles: high salinity. Data obtained 72 h after copper sulfate exposure in cells (left graphs) and culture media (right graphs). At the bottom-center, the absorbance from the different culture media with 0.5 mM of $CuSO_4$ as a reference is shown

35 to 45 nm with a maximum absorbance of 225 nm, while with the media in L+S−, the peak of absorbance was detected at 215 nm with a bump between 235 and 255 nm, showing aggregates with variable morphology in the SEM images.

Discussion

Copper concentration in relation to the reducing and stabilizing agents affects the NP formation (Dang et al. 2011); also, the sensitivity of microalgae to copper sulfate is highly varied among different strains (Levy et al. 2008; Sabatini et al. 2009). The copper ion availability per cell depends on the population of each treatment at the exposure time. This could significantly affect the copper toxicity if lower cell populations are exposed to the same copper concentration (Debelius et al. 2009; Salas-Herrera et al. 2015; Wan et al. 2018). Thus, the copper concentration in each treatment needed to be adjusted based on the strain's initial population, considering 0.5 mM of $CuSO_4$ for the highest populations. However, based on the biovolume (Fig. 4c), the highest copper concentration was in treatments with *T. suecica*, resulting in the culture bleaching in all treatments after 96 h of exposure. With *D. tertiolecta*, the bleaching was detected only in the low-salinity treatments, and *C. kessleri* had the lowest copper concentration, without an apparent mortality being detected.

The survival detected for *D. tertiolecta* in treatments of high salinity (1.5 M NaCl) is interesting because, despite being under osmotic stress in combination with both illuminations (high and low), the toxic load of copper in the culture media weathered better in comparison with the low-salinity treatments (0.46 M). The salt stress induces *Dunaliella salina* to the secretion of polymeric substances (Liu et al. 2016), which could reduce the copper availability in the culture media. Also, it has been reported that high copper concentrations modify the metabolism of *D. tertiolecta*, releasing phenolic compounds to the media (López et al. 2015). Copper internalization by three strains, including *D. tertiolecta* and *Tetraselmis* sp., was evaluated by Levy et al. (2008); they found that *D. tertiolecta* is more efficient to the exclusion of copper, lowering the internalization rate and keeping the intracellular concentration three times lower than *Tetraselmis* sp., and concluded that copper detoxification mechanisms are intracellular in *Tetraselmis* sp. and extracellular in *D. tertiolecta.* In the case shown in this study, it could be that some biomolecules secreted to the culture media by *D. tertiolecta* avoided the copper toxicity under high-salinity conditions. The low growth of *C. kessleri* and *D. tertiolecta* in

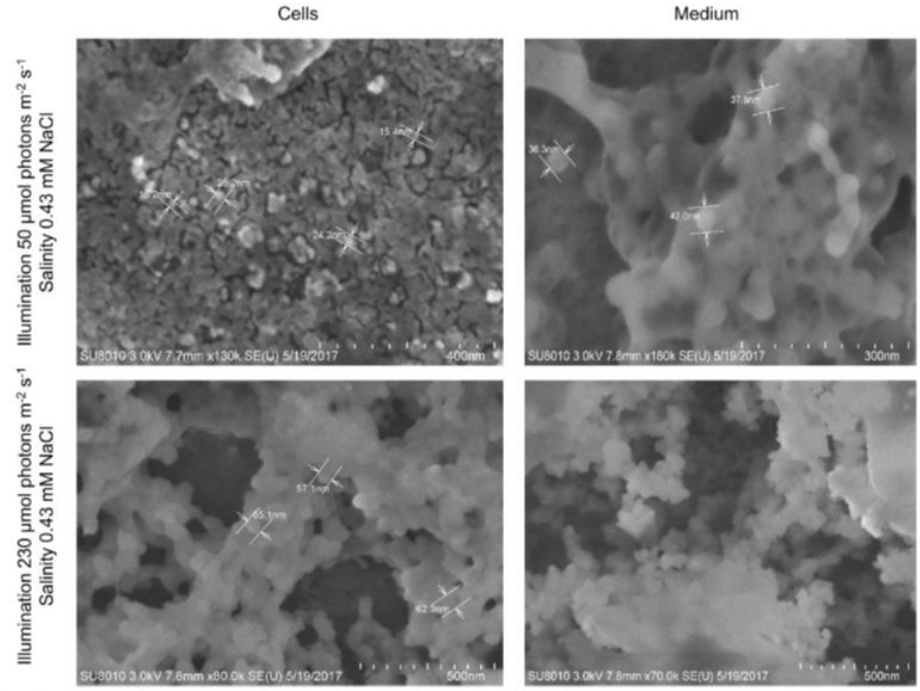

Fig. 7 Scanning electron microscopy (SEM) images of the synthetized nanoparticles in *C. kessleri* treatments

the treatment of L−S+ could be related to the low PAR irradiation, generating a deviation in the energy produced by photosynthesis to deal with the salt stress. The secretion of Na^+ in halotolerant algae such as *D. salina* is done through Na^+/H^+ antiporter pumps and at the expense of energy through a Na^+-ATPase pump (Gimmler 2000), by which salt stress represents a metabolic extra load for the energy produced by photosynthesis.

The exposure to copper sulfate was done at 18 days of culturing. At this time, *D. tertiolecta* (all treatments) and *T. suecica* (L−S− and L+S−) reached the maximum population and were in the stationary growth phase (Fig. 2). At this growth phase, there might be changes in the physiological state of these microalgae, such as a decrease in the protein content (Barbarino and Lourenço 2005) and an increase of triacylglycerides or starch as a store of energy (Davidi et al. 2012). Thus, the results obtained for these treatments may vary considerably if the biosynthesis will be held in the growth phase. This has been proven with *Escherichia coli* by Li et al. (2011) who found that, at the stationary phase of growth, the formation of CdS nanocrystals increased 20 times; at this same stage, extracellular gold nanoparticles were formed by *Trichothecium* sp., but with constant shaking, the biosynthesis tendency was intracellular (Shah et al. 2015).

The culture conditions were selected to cause stress by salinity and illumination for the evaluated strains and thus determine if the stress at which microalgae are exposed promotes the biosynthesis of NPs. In the present study, Cu_n NP formation was detected in the L+S− treatments with cells of the three strains evaluated. Light effects on the biosynthesis of NPs have been reported by several authors; with diatoms, the intervention of photosynthetic pigments as a reducing agent in the formation of silver NPs has been reported (Jena et al. 2015) and the electron transport system in the chloroplasts of plants has been related to gold NP formation (Shabnam and Pardha-Saradhi 2013). However, culture conditions in which Cu_n NPs was observed had an equal or lower photosynthetic pigment content in comparison with the other treatments where the biosynthesis was not detected (Fig. 3). On the other hand, Patel et al. (2015) reported the biosynthesis of silver NPs under light conditions, with microalgae cells as well as with the culture media in which they grow, without detecting the formation when the reaction was done in the dark. Brahmachari et al. (2014) reported silver NP biosynthesis with leaf extracts from *Ocimun sanctum*, where the biomolecules which participated in the reduction of the metallic ions required the activation of sunlight, assuming a mechanism where the phenolic bond O–H undergoes homolytic cleavage under sunlight radiation to form hydrogen radicals, which reduces the silver ions to form Ag NPs.

Microalgae are a source of a variety of compounds which could participate in metallic ion reduction (Siddiqi and Husen 2016). However, the only strain which showed Cu_n NP formation with cells in treatments apart from L+S− was *T. suecica*. With this strain, Cu_n NP formation was also detected in treatments with cells in L−S− and L−S+ conditions. Some microalgae have the capacity to internalize copper, such as for both *T. suecica* and *D. tertiolecta*, increasing the number and size of their vacuoles (Levy et al. 2008). The intracellular detoxification mechanisms could support the hypothesis that biosynthesis with this strain is done by an intracellular mechanism. In the case of *D. tertiolecta*, some biomolecules secreted to the media under salt stress could have prevented the toxicity of copper to the cells. If so, that biomolecule did not favor the formation of Cu_n or CuO nanoparticles which were detectable by UV-vis compared with the low-salinity treatments.

Copper oxide nanoparticles are thermodynamically more stable than pure copper NPs under ambient atmospheric pressure and temperature without proper protection during their biosynthesis (Dang et al. 2011). Oxygen when combined with copper forms cuprous oxide (Cu_2O), cupric oxide (CuO), and copper dioxide (CuO_2), which could form hexagonal patterns, alternating copper with the oxygen atoms (Subramanian et al. 2015). The media, in which the treatments were realized with photosynthetic organisms exposed to $CuSO_4$ under light conditions, supposes a high oxygen environment at the reaction time. Moheimani (2013) reports values from 1 to 16 mg L^{-1} of O_2 from early in the morning to 5 h later in photobioreactors with *D. tertiolecta* and *Chlorella* sp. and daily pH fluctuations from 6.5 to 9.8. These conditions could produce the oxidation of the formed copper nanoparticles. Rahman et al. (2009) monitored the oxidation of Cu_2O NPs biosynthesized with the biomass of a cyanobacterium of the genus *Phormidium* with constant agitation under aerobic conditions, finding that, after 36 h of reaction time, a considerable amount of Cu_2O had oxidized to CuO, disappearing the signals of Cu_2O after 48 h and increasing the intensity of absorbance of CuO. The absorbance curves in the present report were taken 72 h after the reaction started, and so the presence of Cu_2O NPs prior to the formation of CuO NPs cannot be ruled out.

The shift in the wavelengths of maximal absorption in the treatments were CuO NPs was found to be possibly related to the different sizes found (Bouazizi et al. 2015). Dang et al. (2011) comment that the increase in the bandwidth of the resonance with the decreasing size of the NPs is due to the increased dispersion of electrons on their surface, which could be a tool to the monitoring of NP formation. The biosynthesis of NPs with the media in which microalgae were cultured may have different biomolecules involved in reducing and stabilizing the metal ions than those involved in the presence of cells, and so there may be variations in the maximum absorbance apart from those caused by the size of the NPs formed (Shantkriti and Rani 2014). Illumination in combination with the salinity of the culture media affects microalgae, and this affects the biosynthesis of NPs not only in terms of their size and conformation but in the possibility of obtaining them.

Conclusions

Copper nanoparticle biosynthesis with microalgae has the potential to be controlled based on the strain and the manipulation of illumination and salinity through culturing. Optimizing the culture conditions to produce biomass is not necessarily the best condition for the biosynthesis of copper nanoparticles. *Chlorella kessleri* under autotrophic conditions could be a good option for the biosynthesis of copper oxide nanoparticles by both cells and the culture media in which they were cultured. Copper nanoparticle biosynthesis is most probable with cells cultured at a low salinity (0.43 mM NaCl for *C. kessleri* and 0.46 M NaCl for both *D. tertiolecta* and *T. suecica*) and high illumination (230 μmol photons m^{-2} s^{-1}), while *T. suecica* offers the best chance of success under different culture conditions, except for the case of high salinity (1 M NaCl) and high illumination (230 μmol photons m^{-2} s^{-1}).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abboud Y, Saffaj T, Chagraoui A, El Bouari A, Brouzi K, Tanane O, Ihssane B (2013) Biosynthesis, characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract (*Bifurcaria bifurcata*). Appl Nanosci 4:571–576

Barbarino E, Lourenço SO (2005) An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae. J Appl Phycol 17:447–460

Barwal I, Ranjan P, Kateriya S, Yadav S (2011) Cellular oxido-reductive proteins of *Chlamydomonas reinhardtii* control the biosynthesis of silver nanoparticles. J Nanobiotechnol 9:56

Bouazizi N, Bargougui R, Oueslati A, Benslama R (2015) Effect of synthesis time on structural, optical and electrical properties of CuO nanoparticles synthesized by reflux condensation method. Adv Mater Lett 6:158–164

Brahmachari G, Sarkar S, Ghosh R, Barman S, Mandal NC, Jash SK, Banerjee B, Roy R (2014) Sunlight-induced rapid and efficient biogenic synthesis of silver nanoparticles using aqueous leaf extract of *Ocimum sanctum* Linn. with enhanced antibacterial activity. Org Med Chem Lett 4:18. https://doi.org/10.1186/s13588-014-0018-6

Cirulis JT, Scott JA, Ross GM (2013) Management of oxidative stress by microalgae. Can J Physiol Pharmacol 91:15–21

Dahoumane S, Jeffryes C, Mechouet M, Agathos S (2017) Biosynthesis of inorganic nanoparticles: a fresh look at the control of shape, size and composition. Bioengineering 4:4010014

Dang TMD, Le TTT, Fribourg-Blanc E, Dang MC (2011) Synthesis and optical properties of copper nanoparticles prepared by a chemical reduction method. Adv Nat Sci Nanosci Nanotechnol 2:015009

Davidi L, Katz A, Pick U (2012) Characterization of major lipid droplet proteins from *Dunaliella*. Planta 236:19–33

Debelius B, Forja JM, DelValls A, Lubián LM (2009) Toxicity and bioaccumulation of copper and lead in five marine microalgae. Ecotoxicol Environ Saf 72:1503–1513

Fox J, Weisberg S (2011) An R companion to applied regression. second edition. Sage, Thousand Oaks

García F, Freile-Pelegrín Y, Robledo D (2007) Physiological characterization of *Dunaliella* sp. (Chlorophyta, Volvocales) from Yucatan, Mexico. Bioresour Technol 98:1359–1365

Gawande MB, Goswami A, Felpin FX, Asefa T, Huang X, Silva R, Zou X, Zboril R, Varma RS (2016) Cu and Cu-based nanoparticles: synthesis and applications in catalysis. Chem Rev 116:3722–3811

Gimmler H (2000) Primary sodium plasma membrane ATPases in salttolerant algae: facts and fictions. J Exp Bot 51:1171–1178

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms: I *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

Hafeez A, Razzaq A, Mahmood T, Jhanzab HM (2015) Potential of copper nanoparticles to increase growth and yield of wheat. J Nanosci Adv Tech 1:6–11

Henriques M, Silva A, Rocha J (2007) Extraction and quantification of pigments from a marine microalga: a simple and reproducible method. In: Méndez-Vilas (ed) Communicating Current Research and Educational Topics and Trends in Applied Microbiology. Formatex, Badajoz, pp 586–593

Jena J, Pradhan N, Nayak RR, Dash BP, Sukla LB, Panda PK, Mishra BK (2014) Microalga *Scenedesmus* sp.: a potential low-cost green machine for silver nanoparticle synthesis. J Microbiol Biotechnol 24: 522–533

Jena J, Pradhan N, Dash BP, Panda PK, Mishra BK (2015) Pigment mediated biogenic synthesis of silver nanoparticles using diatom *Amphora* sp. and its antimicrobial activity. J Saudi Chem Soc 19: 661–666

Levy JL, Angel BM, Stauber JL, Poon WL, Simpson SL, Cheng SH, Jolley DF (2008) Uptake and internalisation of copper by three marine microalgae: comparison of copper-sensitive and copper-tolerant species. Aquat Toxicol 89:82–93

Li X, Xu H, Chen ZS, Chen G (2011) Biosynthesis of nanoparticles by microorganisms and their applications. J Nanomater 270974:1–16

Lichtenthaler HK, Wellburn AR (1983) Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochem Soc Trans 11:591–592

Liu L, Pohnert G, Wei D (2016) Extracellular metabolites from industrial microalgae and their biotechnological potential. Mar Drugs 14:191

López A, Rico M, Santana-Casiano JM, González AG, González-Dávila M (2015) Phenolic profile of *Dunaliella tertiolecta* growing under high levels of copper and iron. Environ Sci Pollut Res 22:14820–14828

Mendiburu F (2016) agricolae: Statistical Procedures for Agricultural Research. R package version 1.2–0. http://CRAN.R-project.org/package=agricolae; searched on 25 September 2017

Miazek K, Iwanek W, Remacle C, Richel A, Goffin D (2015) Effect of metals, metalloids and metallic nanoparticles on microalgae growth and industrial product biosynthesis: a review. Int J Mol Sci 16: 23929–23969

Moheimani NR (2013) Long-term outdoor growth and lipid productivity of *Tetraselmis suecica, Dunaliella tertiolecta* and *Chlorella* sp (Chlorophyta) in bag photobioreactors. J Appl Phycol 25:167–176

Mohseniazar M, Barin M, Zarredar H, Alizadeh S, Shanehbandi D (2011) Potential of microalgae and lactobacilli in biosynthesis of silver nanoparticles. BioImpacts 1:149–152

Morales-Díaz AB, Juárez-Maldonado A, Morelos-Moreno Á, González-Morales S, Benavides-Mendoza A (2016) Biofabricación de nanopartículas de metales usando células vegetales o extractos de plantas. Rev Mex Cienc Agríc 7:1211–1224

Patel V, Berthold D, Puranik P, Gantar M (2015) Screening of cyanobacteria and microalgae for their ability to synthesize silver nanoparticles with antibacterial activity. Biotechnol Rep 5:112–119

Pestryakov AN, Petranovskii VP, Kryazhov A, Ozhereliev O, Pfänder N, Knop-Gericke A (2004) Study of copper nanoparticles formation on supports of different nature by UV-visible diffuse reflectance spectroscopy. Chem Phys Lett 385:173–176

Rahman A, Ismail A, Jumbianti D, Magdalena S, Sudrajat H (2009) Synthesis of copper oxide nano particles by using *Phormidium* cyanobacterium. Indo J Chem 9:355–360

Raposo MFJ, De Morais RMSC, De Morais AMMB (2013) Bioactivity and applications of sulphated polysaccharides from marine microalgae. Mar Drugs 11:233–252

Sabatini SE, Juárez AB, Eppis MR, Bianchi L, Luquet CM, Ríos de Molina MDC (2009) Oxidative stress and antioxidant defenses in two green microalgae exposed to copper. Ecotoxicol Environ Saf 72:1200–1206

Salas-Herrera G, Benavides-Mendoza A, Zermeño-González A, Orta-Dávila A, Sánchez-Pérez FJ (2015) Evaluación de microalgas para la producción de biomasa económicamente útil usando aguas producidas. Rev Mex Cienc Agríc 12:2423–2435

Shabnam N, Pardha-Saradhi P (2013) Photosynthetic electron transport system promotes synthesis of Au-nanoparticles. PLoS One 8: e71123

Shah M, Fawcett D, Sharma S, Tripathy SK, Poinern GEJ (2015) Green synthesis of metallic nanoparticles via biological entities. Materials 8:7278–7308

Shantkriti S, Rani P (2014) Biological synthesis of copper nanoparticles using *Pseudomonas fluorescens*. Int J Curr Microbiol App Sci 3: 374–383

Shobha G, Moses V, Ananda S (2014) Biological synthesis of copper nanoparticles and its impact—a review. Int J Pharm Sci Invent 3: 28–38

Siddiqi KS, Husen A (2016) Fabrication of metal and metal oxide nanoparticles by algae and their toxic effects. Nanoscale Res Lett 11: 363–363

Simionato D, Sforza E, Corteggiani Carpinelli E, Bertucco A, Giacometti GM, Morosinotto T (2011) Acclimation of *Nannochloropsis gaditana* to different illumination regimes: effects on lipids accumulation. Bioresour Technol 102:6026–6032

Singh Sekhon B (2014) Nanotechnology in agri-food production: an overview. Nanotechnol Sci Appl 7:31–53

Singh J, Kaur G, Rawat M (2016) A brief review on synthesis and characterization of copper oxide nanoparticles and its applications. Nanotechnol Sci Appl 1:1–9

Subramanian S, Valantina R, Ramanathan C (2015) Structural and electronic properties of electrodeposited heterojunction of CuO, CuO_2 and Cu_2O nanoclusters a DFT approach. Mater Sci 21:173–178

Sudha SS, Rajamanickam K, Rengaramanujam J (2013) Microalgae mediated synthesis of silver nanoparticles and their antibacterial activity against pathogenic bacteria. Indian J Exp Biol 52:393–399

Sun J, Liu D (2003) Geometric models for calculating cell biovolume and surface area for phytoplankton. J Plankton Res 25:1331–1346

Suresh Y, Annapurna S, Bhikshamaiah G, Singh AK (2016) Green luminescent copper nanoparticles. J Bioelectron Nanotechnol 149: 012187

Team RS (2016) RStudio: Integrated Development for R. RStudio, Inc, Boston, MA. http://www.rstudio.com; searched on September 2017

Valli G, Suganya M (2015) Green synthesis of copper nanoparticles using *Cassia fistula* flower extract. J Bio Innov 4:162–170

Wan J-K, Chu W-L, Kok Y-Y, Cheong K-W (2018) Assessing the toxicity of copper oxide nanoparticles and copper sulfate in a tropical *Chlorella*. J Appl Phycol 30:3153–3165

SEGUNDO ARTÍCULO

Interaction areas in the microalgae production using wastewater

Interaction areas in the microalgae production using wastewater

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Abstract

Microalgae have a great biotechnological interest for the products that can be obtained from them from lipids, carbohydrates, proteins, and other metabolites. However, large volumes of water and nutrients are required for its production. Thus, wastewater has great potential as a culture medium. The use of this resource represents a positive impact on the environment by using the dissolved nutrients for the development of the microalgae, as well as by the sequestration of atmospheric CO_2 through the photosynthetic activity. In view of this, the urban wastewater treatment plants represent an opportunity for this purpose. However, there are a number of factors that have limited the establishment of these systems on a large scale. Therefore, in the present work, the advances for the production of microalgae biomass with the use of urban wastewater were analyzed. The interaction of three areas was taken into account: 1) wastewater, its characteristics and variations in the different stages and processes for its treatment, 2) microalgae and some considerations for the selection of strains, and 3) photobioreactor engineering and its possible incorporation to wastewater treatment. In general, some critical points are detected that could be useful in future investigations and the development of wastewater systems for the cultivation of microalgae.

Key words:

Wastewater treatment, bioremediation, urban wastewater, biotechnology, photobioreactor.

Introduction

The treatment and reuse of wastewater represents a strategy for the preservation of the quality of natural waters (Sala and Mujeriego 2001) since the contamination of this resource is a real problem for the current global population (Onda et al. 2012). The treatment of wastewater with the cultivation of microalgae allows the use of pollutants such as nitrogen and phosphorus (Sriram and Seenivasan 2012) when using them as nutrients for their cultivation, decreasing its content. It also has the potential to treat other highly toxic pollutants, such as heavy metals (Das et al. 2009; Dwivedi 2012) and radioactive elements (Potera 2011; Fukuda et al. 2014), depending on the source of the contaminated water that is to be treated.

There is a wide field of research regarding the bioremediation of water using these microorganisms. On the one hand, there are studies that focus on the treatment of contaminated or wastewater as the main objective (de-Bashan y Bashan 2010; Hii et al. 2011), either using microalgae in conjunction with bacteria or fungi, or using biomass as a heavy metal adsorbent material (Dwivedi 2012). On the other hand, there are studies where the treatment of wastewater is sought through its use for the production of biomass useful for other purposes (Christenson and Sims 2011). The result is a positive impact on the environment, as well as a profitable activity for the products that could potentially be obtained. This would partially reduce the pressure on this appreciable resource.

The establishment of a system for the production of microalgae through the treatment and use of wastewater seeks to take advantage of the nutrients dissolved in them. However, wastewater from various anthropogenic activities are varied in composition, physicochemical characteristics, volume, and periodicity. Taking into account that domestic wastewater has little variation in its components (Noyola 2013), as long as it is not mixed with rainwater or industrial processes, effluents from urban water treatment plants have been considered as a valuable means for the production of biomass with microalgae (Cabanelas et al. 2013). On the other hand, there are other sources of contaminated water that are currently being used for the production of microalgae, such as those from the gas extraction industry (Sullivan et al. 2012; Hamawand et al. 2014; Salas-Herrera et al. 2015), for which it is very important to know the characteristics of the water and its possible variations during its use, as well as the capacities and limitations of the strains selected for it.

The term microalgae refer to photosynthetic microorganisms with varied morphological, physiological, and reproductive characteristics. Some are considered cyanobacteria and eukaryotic microalgae, among which are: diatoms, green algae (Chlorophyta), red (Rhodophyta), and brown (Phaetophyta), developing in a wide variety of habitats and ecological niches. Some are considered cyanobacteria and eukaryotic microalgae, among which we are: diatoms, green algae (Chlorophyta), red (Rhodophyta), and brown (Phaetophyta) (Brooijmans and Siezen 2010), develop in different habitats and ecological niches. Different species require different nutrient ratios for their reproduction and some species can reproduce in a wide range of nutrient concentrations (Agrawal, 2012). In general, when the environmental conditions are unfavorable, there are heavy metals or chemical products that can induce an oxidative stress that affects their development (Cirulis et al. 2013). However, selective pressure in contaminated water can lead to physiological and genetic adaptations through spontaneous mutations (Carrera-Martinez et al. 2011). Therefore, it is important to know the physiological and adaptive capacities of the strains of interest, as well as the characteristics and variability of wastewater for its use.

The production of microalgae for different purposes (food, metabolites, biofuels, among others) implies the search for optimal conditions for their development in spaces designed for this purpose. This design should favor environmental conditions for the proper development of microalgae (lighting, turbidity, temperature, hydraulic flow), as well as supplement their nutritional needs. Among the needs of microalgae that most influence the design of photobioreactors is lighting, this has been fulfilled using designs that shorten the distance that light travels in the water column, combined with good turbulence of the culture medium decreases the autosomal, guaranteeing good exposure to the light of most cells. These designs, along with the harvest costs, affect the profitability of the process and can only be justified with the value of the biomass obtained. On the other hand, there are open systems such as the HRAP system (High rate algal pond system), and fixed or biofilm culture systems, which seek to reduce these costs. For the use and treatment of wastewater with microalgae, several points have to be considered. The metabolic capacities of microalgae in relation to the characteristics of the wastewater in its different stages of traditional treatment, the identification of the stages with the greatest potential for its use, the technological and operative viability for the adaptation of the microalgae culture and the use of technological advances in the design and construction of photobioreactors. In the present work, the objective was to show the advances that have been made regarding the use of wastewater for the production of biomass with microalgae. The identification of critical points for the establishment of an efficient system, as well as the advances or possible solutions for it. For this, the interaction of three major themes was analyzed; microalgae, wastewater, and engineering developed for this purpose (Fig 1).

Wastewater Treatment

In order to use wastewater for the production of microalgae, it is important to know the characteristics of these waters and the expected changes in each phase of their treatment. Water pollution is related to its use and bears the traces of the processes in which it was used. Water is the main resource to maintain the hygiene and health of human beings and their environment (Hunter et al. 2010), in the preparation of food (from the field to the table), and is an indispensable resource in a great diversity of industrial processes (Cassardo and Jones 2011). The load of contaminants that it carries is directly related to these processes. The parameters most used to characterize the degree of contamination before and after entering a treatment process are: biological oxygen demand, chemical oxygen demand, total suspended solids, pH, total phosphorus, and total nitrogen. Other important components are inorganic compounds, heavy metals, fats and oils (Environmental Protection Agency 1997). The appearance of new pollutants such as drugs, personal care products and pesticides among others must be added (Matamoros et al. 2015). The purpose of wastewater treatment is to minimize the footprint of its use. Within this process, the points of greatest value must be detected to integrate the production of microalgae.

Wastewater treatment is divided into several stages that combine physical-chemical and biological processes. In general, we find three phases in addition to the preliminary treatment and treatment of sludge (Fig 2). The preliminary treatment consists of the removal of larger components (plastics, branches, dead animals, sands), which can affect the operation in the following stages. On the other hand, sludge is formed by the precipitation of suspended solids in the primary treatment and by the production of biomass by the microorganisms in the secondary treatment. These are separated from the water flow and are treated separately, some of the processes for their treatment are: anaerobic digestion, aerobic digestion, composting with cellulose residues, lime stabilization, incineration, and pasteurization (Noyola et al. 2013).

The primary treatment consists in the precipitation of suspended solids by gravity by decreasing the speed of the water flow. About 60% of the suspended solids are removed and the biological demand of oxygen is reduced by about 35% (Environmental Protection Agency 1997; Noyola et al. 2013). In the secondary treatment, by means of biological processes the biodegradable organic matter is eliminated, these processes occur under aerobic, anaerobic or combined conditions. The organic matter assimilated by aerobic processes produces biomass that precipitates in the form of flocs, water, CO_2 and energy in the form of heat. With anaerobic processes, the main product is biogas with a low biomass production (Adekunle and Okolie 2015). The treatment lagoons, also known as facultative lagoons, depend on the photosynthetic processes of algae. They use nitrogen, phosphorus and CO_2 to produce biomass and oxygen that is used in aerobic digestion processes, degrading organic compounds and generating more CO_2 and nutrients that are used by algae (Craggs et al. 2012). The tertiary treatment consists of the removal of nitrogen, phosphorus, and some organic compounds (Razzak et al. 2013).

During the different phases of treatment, not only pollutant compounds are eliminated. The characteristics of the remaining organic matter change, as well as the chemical forms of nitrogen and phosphorus (Maurer and Boller 1999; Paredes et al. 2007; Wang and Chen 2018). Phosphorus, which occurs in various forms (particulate, orthophosphate, polyphosphate, and organic phosphorus), not only decreases its concentration during the course of treatment but the relationship between the dominant form, after primary treatment there is a greater proportion of orthophosphate and polyphosphates; after tertiary treatment there is a dominance of organic phosphorus (Maurer and Boller 1999). In traditional treatments, nitrogen in the form of ammonium is converted to nitrates via nitrites in the nitrification process. In a second stage during the denitrification process, it is reduced to gaseous nitrogen by numerous heterotrophic bacteria that use dissolved organic carbon (DOC). The efficiency of this process is dependent on the N/DOC ratio, the biodegradability of the DOC and the concentration of dissolved oxygen. In general, the initial form in which we find nitrogen at the beginning of the treatment is as ammonium and the final products are nitrogen gas and nitrate (Paredes et al. 2007). However, some forms of dissolved organic nitrogen persist.

Dissolved organic matter represents between 82.6 and 86.6% of the total organic carbon. The secondary treatment effluent contains a considerable amount of products associated with biomass, including microbial products of slurries with a high molecular weight. Dissolved organic matter can degrade and increase the amount of biodegradable organic carbon in advanced treatment with processes such as UV/H_2O_2 radiation by changing the bioavailability of nitrogen and dissolved organic phosphorus influencing the growth of algae in the effluent receptor (Wang y Chen 2018). On the other hand, ozonation of the effluent causes the discoloration of the organic matter increasing the transparency of the water (Wenk et al. 2015). Therefore, different strategies in the treatment of the final wastewater effluent affect in different ways the final composition of the dissolved organic matter.

Problems with microalgae in wastewater

The wastewater flows are subject to variations in time scales of hours, weeks, and season of the year (Environmental Protection Agency 1997), in addition, the use of drainage systems combined with rainwater and the incorporation of industrial drainage (food industry, livestock, metalworking, chemistry, etc.) alter the concentration of normally expected compounds with the risk of incorporating harmful toxic substances to the treatment of the effluent (Noyola et al. 2013) and causing variations in the concentration and relationship between the expected nutrients. Zhang et al. (2016) found that the bioavailability of dissolved organic nitrogen was lower when the wastewater contained 40% of water from industries compared to 100% urban water. On the other hand, the authors comment that during the anaerobic process some metal complexes combined with organic matter can be released by aerobic bacteria to their ionic form causing negative effects in the cellular physiology of microalgae. Therefore, it is important to identify the possible risks related to the influence of wastewater from activities unrelated to domestic use that are discharged into the collection system.

The presence of heavy metals in the wastewater could be a limiting factor for its use in the production of biomass with microalgae. Although some heavy metals are essential as micronutrients (copper and zinc), in high doses they can be toxic, and the presence of metals such as mercury, lead, arsenic, or cadmium could cause toxic effects in microalgae (Dong et al. 2014). The solutions obtained from the thickening of activated sludge and anaerobic digestion contain a high concentration of nutrients, however, they also concentrate substances (such as heavy metals) that can be harmful for the development of microalgae (Guldhe et al. 2017). This increase in the concentration of metals has been reported in sludges from different stages of wastewater treatment (Healy et al. 2016). The use of these waters rich in nutrients from the thickening of sludge has been sought through its dilution in waters of primary (Caporgno et al. 2015) or secondary treatment (Álvarez-Díaz et al. 2017) in laboratory conditions (Ge et al. 2018; Maeng et al. 2018a; Maeng et al. 2018b).

Urban wastewater contains chemical compounds related to personal care, hygiene, health and cosmetics, as well as products commonly used in the home such as disinfectants, surfactants and anticorrosive agents. The treatment of some of these products in the cultivation of microalgae with wastewater has already been evaluated. In a literature review on the removal of pharmaceutical contaminants with microalgae, Xiong et al. (2018), conclude that the integrated treatment of algae-based technologies in conjunction with advanced oxidation processes would be a feasible option for the advanced remediation of new organic contaminants such as pharmaceutical products. The main focus of some of these studies is directed to the treatment of these products within a comprehensive water treatment system considering the intervention of microalgae as part of this process (Gutiérrez-Alfaro et al. 2018). On the other hand, de Wilt et al. (2016), evaluating the treatment of six pharmaceutical products and three estrogens, reported that the presence of these products did not inhibit the growth of *Ch. Sorokiniana*. However, the presence of these, and that of new pollutants in wastewater should be considered for the production of microalgae.

Due to its nature and characteristics, wastewater contains a variety of microorganisms and larger organisms that can interfere with the development of microalgae in them, from microalgae, bacteria or fungi, to herbivore species that can consume the microalgae of the culture (Guldhe et al. 2017). The decrease of turbidity and the elimination of organisms is one of the reasons why some studies in which they study the interaction of microalgae with waste water filter it before exposure (Henkanatte-Gedera et al. 2017; Ferro et al. 2018; Hughes et al. 2018). On a larger production scale, the most viable options are; ozonation, disinfection with ultraviolet rays, chlorination, and acidification (Guldhe et al. 2017). Open culture systems are subject to the invasion and proliferation of herbivore species of zooplankton (rotifers, ostracods, copepods, and daphnia). Asphyxia by addition of CO_2 at night effectively controls the density of zooplankton and increases the production of biomass by microalgae (Montemezzani et al. 2017a). On the other hand, the phototaxis that some of these organisms present allows to concentrate them in the upper layer of the water column, which can facilitate the filtration of a smaller volume of water for their separation (Montemezzani et al. 2017b).

The geographical location and climatic characteristics of the exploitation site are factors that affect the environmental conditions of the microalgae culture. In medium latitudes with temperate climates, considerable temperature variations occur throughout the year. This means that there are different yields in the production of biomass with microalgae, or that different strategies have to be taken depending on the season of the year. Osundeko and Pittman (2014), reported the culture throughout the year in an open system with water obtained from a wastewater treatment plant in England. In their work they found that *Chlorella luteoviridis* and *Parachlorella hussii*, can develop throughout the year, however, the best results in terms of obtaining biomass and removal of nutrients are recorded in the summer and spring. On the other hand, the use of heat generated in other industrial processes can be an option to increase the temperature of the cultivation system in regions with cold seasons or climates (Ekendahl et al. 2018), which requires special adaptations or the use of the energy that could be generated by the production of biogas with sludge from wastewater treatment.

Metabolic capacities and strain selection

Possibly one of the most striking reflections of the relationship between wastewater and microalgae is in the processes of eutrophication of aquatic ecosystems affected by discharge of drainages with high concentrations of nitrogen and phosphorus. The process begins with an increase in nutrients in the body of water, an uncontrolled increase in primary products and the subsequent accumulation and decomposition of organic matter, causing a series of subsequent problems (Sala and Mujeriego 2001). Among the symptoms of an aquatic system in eutrophication is the massive proliferation of harmful and dangerous microalgae for aquatic organisms as well as for humans (Rabalais et al. 2009). Therefore, in a microalgae production system using wastewater, it is sought to replicate this phenomenon under controlled conditions and with strains of interest for the products that could be obtained from the biomass generated. Delrue et al. (2016), refer to this possibility of having a positive environmental impact, obtaining a profitability with the biomass produced as the paradigm of win-win. For such a system to be real, special attention must be paid to all the details, starting with the strains of microalgae to be used.

Some of the factors to be considered in the selection of strains for their development in wastewater depend on the characteristics of the waters with which they are going to work and the climatic characteristics where the water treatment is to be carried out. Different species of microalgae find the optimal conditions for their development in different environmental conditions (Olguín 2012). In general, the desired characteristics are; the efficiency in nitrogen and phosphorus removal as well as other contaminants that can be cultivated on a large scale, a high growth rate and tolerance to variations in temperature, salinity and nutrient availability. On the other hand, the value of the biomass produced, which can be allocated according to its composition and quality for animal feed, fertilizers, soil improvers, bioplastic materials or for the production of energy (Christenson and Sims 2011).

There are collections of microalgae such as the University of Texas (UTEX) with more than 3,000 strains available (www.utex.org). However, taking into account that wastewater could represent an adverse culture medium, the search for strains in fluctuating natural environments (temperature, lighting, salinity, availability of nutrients) such as intertidal zones, rivers or temporary ponds could represent the selection of strains with better survival strategies. Duong et al. (2012), recommend this search for oleaginous microalgae species to produce biodiesel. In this context, Sasongko et al. (2018) used a polyculture of native strains in Japan (dominated by the genus *Desmodesmus* sp and *Scenedesmus* sp), using wastewater as a culture medium in a pilot plant for the production of microalgae oils, demonstrating the potential of this selected strains.

The selection of strains that reproduce in zones with the presence of pollutants and have been subject to strong selection pressure, could have presented mutations that would give them an extra advantage over organisms of the same species developed under conditions free of pollutants. This has been found in the case of contamination with hydrocarbons (Romero-López et al. 2012; Carrera-Martinez et al. 2011), as well as by continuous exposures over long periods of time. This opens up the possibility of obtaining strains of microalgae with robust adaptive capacities to deal with the possible variability in the composition of wastewater. The increase of some element in the environment could be interpreted as the scarcity of another (Agrawal 2012), thus the addition of one nutrient could induce the limitation of another, requiring an increase in both nutrients to obtain a substantial increase in biomass (Mackey et al. 2009). Higher-level natural systems (oceans and seas) have much more complex responses at scales of long periods of time compared to small lakes, where normally the main nutrient that limits primary productivity is phosphorus. N: P ratios of 11:1 to 20:1 appear to be favorable for phytoplankton (eukaryotes) while lower ratios favor the blooming of atmospheric nitrogen-fixing cyanobacteria (Hecky and Kilham 1988).

In the treatment with microalgae of the urban wastewater or from the agricultural industry, the main objective is the removal of nitrogen and phosphorus. A more efficient removal of these nutrients in wastewater has been reported when the ratio between nitrogen and phosphorus is from 5:1 to 8:1 (Makareviciene et al. 2013). However, this ratio can be variable depending on the strain and culture conditions. Murwanashyaka et al. (2017) found an efficiency in the removal of nitrogen and phosphorus above 99% with *Chlorella sorokiniana* under heterotrophic culture conditions with an N:P ratio of 10.2:1. When increasing this ratio, the efficiency in nitrogen removal was lower, not phosphorus, so the authors comment that a nitrogen sufficiency is required for the effective removal of phosphorus, but not vice versa, however, nitrogen and phosphorus can be efficient. removed in an optimal ratio of N/P. The imbalance or deficiency of nutrients that can be found in the secondary effluent of wastewater treatment, could be compensated by mixing it with the supernatant of the sludge thickening (Osundeko and Pittman 2014; Álvarez-Díaz et al. 2017; Arias et al. 2018). This represents the need to monitor these effluents based on the availability of nutrients, the needs of the strains in development and whether the production system is autotrophic, mixotrophic, or heterotrophic.

Carbon dioxide is the main source of carbon for the development of microalgae under autotrophic conditions, however, there are species that can take advantage of organic carbon sources, being able to develop under mixotrophic or heterotrophic conditions (Maeng et al. 2018a), which allows the use of some organic carbon compounds dissolved in wastewater. Among the strains that in recent work have been reported to be isolated and develop well in wastewater, are mainly the genus *Chlorella* sp. and *Scenedesmus* sp. (Gouveia et al. 2016; Arias et al. 2018; Ferro et al. 2018) as well as *Chaetophora* and *Naviculata* (Gouveia et al. 2016), *Stigeoclonium* (Arias et al. 2018), *Desmodesmus* and *Coelastrella* (Ferro et al. 2018). Some of these strains have the capacity to develop under phototrophic, mixotrophic or heterotrophic conditions, which allows having a wider range of possibilities in terms of the characteristics of the effluent to be used and the type of bioreactor to be used.

In his review work on the interaction between microalgae and microorganisms for the remediation of wastewater and production of biofuels, Hu et al. (2018), comment in their conclusions that to avoid the negative impact of the natural populations of microorganisms in wastewater, the interactions between microorganisms and microalgae should be optimized through the assembly of artificial consortiums. They also recommend prioritizing the functional diversity of said consortium. Thus, the efficiency in the removal of nutrients and the stability of the crop could be favored, and there is a need for future research in this regard.

Photobioreactor technology

The use of photobioreactors for the production of microalgae, suppose a space where the optimal conditions for their development can be given. The population growth rate in a microalgae culture is affected by environmental parameters such as temperature, dissolved nutrients, light intensity and photoperiod. Lighting and photoperiod are critical components for the development of phototrophic microalgae culture (Wahidin et al. 2013). The intensity of the light through the cultivation of microalgae decreases exponentially as it distances itself from the illuminated walls of the photobioreactors. This decrease is due to the turbidity caused by the growing biomass, metabolites in the environment and biofilm formations in the walls of the photobioreactor (Chen et al. 2011). Due to this, the depth in a photobioreactor design is limited to a few decimeters of the light path in the water column.

The best source of light for the cost and availability is natural light. Of the radiation that reaches the surface of the earth, around 50% corresponds to the photosynthetically active radiation, (wavelength 400 to 700 nm) and from this, the effective conversion to biomass in a microalgae culture corresponds to approximately 5% (Gordon and Seckbach 2012). Direct sunlight is very intense, so for it to be completely used by the microalgae and the excess energy absorbed by the cells is dissipated in the form of fluorescence or heat. Prolonged exposure to high irradiance can overload the energy dissipation mechanisms of cells resulting in photoinhibition and cell damage (Christenson y Sims 2011). Sforza et al. (2012), studied the effects of light on *Nanochloropsis salina* cultures, finding that between 5 and 150 μmol photons $m^{-2}s^{-1}$ increased the growth rate with the increase in lighting, however, above 150 μmol photons $m^{-2}s^{-1}$ the increase in lightning (350 and 1000 μmol photons $m^{-2}s^{-1}$) showed a decrease in population growth. On the other hand, they report that at light intensities of 1000 μmol photons $m^{-2}s^{-1}$ the growth rate was similar to that obtained at 350 μmol photons $m^{-2}s^{-1}$, which indicates that the cells can maintain their protection system and still continue with the population growth. The use of sunlight for the cultivation of microalgae is usually used for open systems or outdoor photobioreactors. The disadvantages are related to the variation in light intensity depending on the time of day, season and weather conditions, as well as exposure to environmental variations.

In indoor cultures with more controlled environmental conditions, the most commonly used lighting is artificial (Ting et al. 2017). This increases the operating costs by the energy expenditure required in the lighting. For a 40 L photobioreactor, it is estimated that 40.32 kW h^{-1} are required with conventional light at 20.16 kW h^{-1} with led light, these costs can be reduced with the combined use of optical fiber and the production of electrical energy by solar cells or energy wind (Chen et al. 2011). Another important aspect to consider in the use of artificial light is its quality, since different sources of artificial light offer different spectra within the PAR radiation, which can positively or negatively affect the population growth of the microalgae culture (Chang et al. 2011). On the other hand, the control of the quality of the light and the use of monochromatic light (blue and red) allows obtaining different metabolic responses Kim et al. (2014) found differences in the concentration and saturation of fatty acids with *Nannochlorpsis gaditana* by varying the quality of light in cultures. With the use of artificial light, the photoperiod is easily controlled along with its effects on crop development (Agrawal 2012; Alkhamis and Qin 2013). Therefore, the control of the intensity, quality of light and photoperiod allow the operation of more efficient photobioreactors for the production of biomass and metabolites. The disadvantages of artificial light are mainly due to the energy expend and the investment required in infrastructure. Open systems without agitation are the closest thing to the natural system, have low cost and are a simple option for commercial scale cultures (Ting et al. 2017), their use is profitable in strains cultures that require extreme conditions such as *Dunaliella salina*. The location of cultures of this species is usually found in areas with hot, dry weather and in or near a brine source (Shariati and Hadi 2011). The cultivation of Spirulina is another example of these open systems, where the typical size of the ponds is 0.3 to 0.5 Ha (Benemann et al. 2002). On the other hand, the most common systems for large-scale production are high rate algal pond, which have a system of pallets that allow the circulation of water, algae and the mixture of nutrients however, they frequently show low productivity due to the presence of dark areas, inefficient use of CO_2, inefficient mixing and pollution problems (Christenson and Sims 2011).

The generation of turbulence in the water column is another factor that affects the design and consumption of energy in a photobioreactor (Gómez-Pérez et al., 2017). The turbulence in the water column allows the mixture of nutrients, distribution of CO_2 and the cells to move from dark areas to illuminated areas in cycles of light and dark increasing productivity (Gómez-Pérez et al. 2017). When the culture is exposed to high irradiance, these cycles can favor a good growth rate and avoid photoinhibition (Sforza et al. 2012). The mechanisms for the generation of turbulence can be given by an impeller driven by an electric motor as in the case of the "stirred tank reactor" (Tsai et al. 2012), or by a bubble column which it can work as an "air lift" system (Ting et al. 2017). These systems are used in crops where the cells are suspended in the medium, which can represent high costs and technical difficulties when harvesting the biomass (Gutiérrez et al. 2016). These problems have given rise to the new microalgae immobilization systems (Wang et al. 2018), where their configuration and operation change considerably from traditional photobioreactor systems.

The immobilization of microalgae in alginate spheres offers the advantage of easy harvesting of the biomass produced (de-Bashan and Bashan 2010). On the other hand, during the last 10 years, the fixation of microalgae in a substrate for the production of biofilm has gained interest. This is because they represent a considerable reduction in the energy required for their production and harvesting costs (Wang et al. 2018), offering promising alternatives in the production of microalgae biomass.

Current proposals for the design of photobioreactors

Currently, a wide variety of photobioreactor designs can be found for microalgae monocultures. Ting et al. (2017) make an extensive review of these and those that have been reported for the use of wastewater. The authors conclude that there are few legitimate designs for this purpose, the majority being adopted directly from monoculture designs. They add that in the design of photobioreactors for the use of wastewater should be taken into account: the characteristics of wastewater, the basic theory in their treatment and the characteristics of microalgae strains. This coupled with the management of regular parameters such as aeration, lighting, and turbulence. So the advances in design and operation of photobioreactors can be exploited with adaptations to the use of wastewater.

The high rate algae pond (HRAP), has been widely studied for the use of wastewater with the cultivation of microalgae. One of the problems with these systems is the variations in the temperature of the batteries caused by the variations in the environmental temperature. The study of the heat transfer between the ponds and the surrounding environment according to the climatic conditions of the area of interest, can be very useful for the specific design of the ponds in a certain region. The heat loss and evaporation of the ponds can be predicted based on the ratio of the width and depth of the channels, as well as the speed of the stirring blades. These predictions would allow a better design and management of these systems for certain areas (Ali et al. 2017). Likewise, the optimization in the operation of the cultivation ponds (supply rate of fresh culture medium and culture removal) based on the weather forecast coupled with a microalgae productivity model was evaluated by De-Luca et al. (2017), obtaining a productivity 2.13 times higher than that obtained with a constant depth and dilution rate. On the other hand, the difficulty to maintain the predominance of the microalgae strain of interest in these systems is another problem that is sought to be solved. Yun et al. (2018) proposed for this purpose a hybrid system of a photobioreactor with open ponds, the photobioreactor allows the monoculture of the strain of interest on a small scale, but sufficient to provide daily doses of inoculum in open cultures maintaining the predominance of this strain.

Tubular photobioreactors offer a good design for the production of microalgae with high productivity, however, the energy cost required to maintain turbulence can affect profitability. By computer simulation of fluid dynamics, it has been found that the use of turbulence promoters in the walls of the tubular photobioreactor can reduce the energy demand of 60 to 80% (Gómez-Pérez et al. 2015). In a similar way, different torsions of tubular photobioreactors have been analyzed to favor the turbulence by spiral movement, reporting that it could be a good option for the configuration of these systems with high efficiency (Gómez-Pérez et al. 2017). On the other hand, the cultivation of cells in suspension in these systems hinders the separation of the biomass and the culture medium, so that the harvest is linked to the hydraulic retention time. The use of membranes allows the separation of the solid phase from the liquid one, which allows the hydraulic retention to be independently managed to that of the biomass. Through the use of hollow fiber membranes, Gao et al. (2018) found that with a hydraulic retention time of two days, the highest biomass production of *Chlorella vulgaris* is obtained using simulated residual water from the secondary effluent. They also report that the system is efficient for up to 130 days with biomass retention times of 21 days, efficiently removing the nutrient load of the water. Photobioreactor systems of membrane usually combine closed systems (airlift or flat panel) with a submerged membrane (hollow fibers) or in the form of sheets to separate the solid phase from the liquid, allowing a greater productivity of biomass and a removal of nutrients, more efficient than other photobioreactors (Luo et al. 2017).

Porous substrate bioreactors for the production of microalgae through fixed culture have gained great interest in recent years, this by considerably reducing the volume of water required for cultivation, direct exposure to atmospheric carbon dioxide and direct exposure to light. This gives great advantages over suspended crops or submerged biofilm (Podola et al. 2017). Schultze et al. (2015) reported that production can be increased to 31 g of dry weight per m^2 per day with the use of biofilm photobioreactors in parallel sheets (twin-layer biofilm). The authors comment that this is due to the increase in lighting and greater exposure to CO_2 in the environment. The fixed culture of microalgae in different porous or non-porous substrates is another way in which the separation of the hydraulic retention time management of the retention time of the biomass is achieved. Wang et al. (2018), make a revision of these culture systems and classify them according to the design in: photobioreactors of horizontal sheets, vertical sheets, rotary sheets, and radial sheets. Reporting that many of these designs have been evaluated with the use of urban wastewater on a laboratory scale and some on a pilot scale. The authors conclude that this emerging field in the production of microalgae has great potential, both for the production of biomass and for water treatment.

Urban wastewater treatment systems located near coastal areas could be exploited through the OMEGA floating photobioreactor system (Offshore Membrane Enclosure for Growing Algae), offering a series of advantages apart from the saving of land space, such as: temperature stability, energy saving for mixing and temperature control, which is contributed by the body of water that surrounds the system, elimination of evaporation losses and greater CO_2 retention (Harris et al. 2013; Novoveská et al. 2016).

Possible adaptations needed of the current wastewater treatment systems

Some of the studies that have been done to evaluate the cultivation of microalgae using wastewater, have used synthetic medium or with a previous treatment of disinfection or filtration (Caporgno et al. 2015; Henkanatte-Gedera et al. 2017; Murwanashyaka et al. 2017; Maeng et al. 2018a). This is directly related to the specific objectives of these studies. However, it does not mean that filtering or disinfection processes should be included prior to the use of wastewater with the cultivation of microalgae. Although wastewater contains large amounts of microorganisms and protozoans that could have negative effects on the cultivation of microalgae, some species such as *Chlorella sorokiana* could have certain features that allow them to be better candidates to develop in wastewater without prior disinfection processes (Bohutskyi et al. 2015). The use of polycultures and consortia for the production of biomass and wastewater treatment would be preferable to monoculture microalgae. This could reduce the need for the use of expensive filtration and disinfection systems. Which do not guarantee the maintenance of monoculture on a large scale. Novoveská et al. (2016), report the use of filtered urban waste water (70µm mesh) and disinfected (peracetic acid 5-15 ppm) for use in a system of large-scale floating photobioreactors in the coastal zone. They used *Scenedesmus dimorphus*, for the initial inoculum, however, they report that during the course of a year there were consortia of strains in a natural way with variations in the composition of the population of microalgae and a final dominance of the genus *Chlorella* sp.

The space required for the implementation of a microalgae production system with large-scale wastewater is a limiting factor, mainly in large urban centers with high population density. Acién et al. (2016), comment that the application of current technologies for the treatment of wastewater with microalgae is limited to small towns with equivalent discharges between 200 to 15,000 inhabitants. In this sense Buchanan et al. (2018), used a HRAP system incorporated to the wastewater management of a community of 300 people. The system showed satisfactory results in both water treatment and biomass production. On the other hand, treatment systems that use facultative lagoons could be exploited (Christenson and Sims 2011). The HRAPs (High Rate Algal Ponds System) require 50 times more space than the treatments with activated sludge, however, they occupy 5 times less space than the stabilization lagoons (Delrue et al. 2016). Therefore, in a matter of required space, facultative lagoons could be a good option for the production of microalgae using wastewater.

Conclusions

The production of microalgae with wastewater has great potential as part of the treatment, as well as to obtain other valuable products. Some of the aspects that most influence the strategies that could be taken for each specific locality are: the characteristics of the wastewater treatment plants, the climatic variations according to the geographical region and the availability of space. On the other hand, some strategies could be extended, such as the balance of nutrients through the combination of different effluents from the treatment plant, the selection of native strains of the region and the use of polycultures. The concepts of photobioreactors of semipermeable membranes or porous substrates could have great potential by allowing independent management of the hydraulic retention times of the biomass, as well as facilitating harvesting.

However, it is necessary to do more research and development in specific designs for the treatment or use of wastewater.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

References:

Acién FG, Gómez-Serrano C, Morales-Amaral MM, Fernández-Sevilla JM, Molina-Grima E (2016) Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment? Appl Microbiol Biotechnol 100:9013–9022

Adekunle KF, Okolie JA (2015) A review of biochemical process of anaerobic digestion. Adv Biosci Biotechnol 06:205–212.

Agrawal SC (2012) Factors controlling induction of reproduction in algae -review: the text. Folia Microbiol 57:387–407.

Ali H, Cheema TA, Park CW (2017) Numerical prediction of heat transfer characteristics based on monthly temperature gradient in algal open raceway ponds. Int J Heat Mass Transf 106:7–17.

Alkhamis Y, Qin JG (2013) Cultivation of Isochrysis galbana in phototrophic, heterotrophic, and mixotrophic conditions. Biomed Res Int. 983465 doi: 10.1155/2013/983465

Álvarez-Díaz PD, Ruiz J, Arbib Z, Barragán J, Garrido-Pérez MC, Perales JA (2017) Freshwater microalgae selection for simultaneous wastewater nutrient removal and lipid production. Algal Res 24:477–485.

Arias DM, Solé-Bundó M, Garfí M, Ferret I, García J, Uggetti E (2018) Integrating microalgae tertiary treatment into activated sludge systems for energy and nutrients recovery from wastewater. Bioresour Technol 247:513–519.

Benemann JR, Olst JC Van, Massingill MJ, Weissman JC, Brune DE (2002) The controlled eutrophication process: Using microalgae for CO 2 utilization and agricultural fertilizer recycling. Water 2:1433–1438.

Bohutskyi P, Liu K, Nasr LK, Byers N, Rosenberg JN, Oyler GA, Betenbaugh MJ, Bouwer EJ (2015) Bioprospecting of microalgae for integrated biomass production and phytoremediation of unsterilized wastewater and anaerobic digestion centrate. Appl Microbiol Biotechnol 99:6139–6154.

Brooijmans RJW, Siezen RJ (2010) Genomics of microalgae, fuel for the future?: Genomics update. Microb. Biotechnol. 3:514–522.

Buchanan NA, Young P, Cromar NJ, Fallowfield HJ (2018) Performance of a high rate algal pond treating septic tank effluent from a community wastewater management scheme in rural South Australia. Algal Res. 35:325-332

Cabanelas ITD, Ruiz J, Arbib Z, Chinalia FA, Garrido-Pérez C, Fogalla F, Nascimento IA, Perales JA (2013) Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. Bioresour Technol 131:429–436.

Caporgno MP, Taleb A, Olkiewicz M, Font J, Pruvost J, Legrand J, Bengoa C (2015) Microalgae cultivation in urban wastewater: Nutrient removal and biomass production for biodiesel and methane. Algal Res 10:232–239.

Carrera-Martinez D, Mateos-Sanz A, Lopez-Rodas V, Costas E (2011) Adaptation of microalgae to a gradient of continuous petroleum contamination. Aquat Toxicol 101:342–350.

Cassardo C, Jones JAA (2011) Managing water in a changing world. Water 3:618–628.

Chang RL, Ghamsari L, Manichaikul A, Hom EF, Balaji S, Fu W, Shen Y, Hao T, Palsson BO, Salehi-Ashtiani K, Papin JA (2011) Metabolic network reconstruction of Chlamydomonas offers insight into light-driven algal metabolism. Mol Syst Biol 7:518. doi: 10.1038/msb.2011.52

Chen C-Y, Yeh K-L, Aisyah R, Lee D-J, Chang J-S (2011) Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour Technol 102:71–81.

Christenson L, Sims R (2011) Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol. Adv. 29:686–702.

Cirulis JT, Scott JA, Ross GM (2013) Management of oxidative stress by microalgae. Can J Physiol Pharmacol 91:15–21.

Craggs RJ, Benemann J, Lundquist T (2012) Wastewater treatment pond algal production for biofuel. In: Gordon R, Seckbach J (eds) The Science of algal fuels. Springer Netherlands, Dordrecht, pp 425–445

Das BK, Roy A, Koschorreck M, Mandal SM, Wendt-Potthoff K, Bhattacharya J (2009) Occurrence and role of algae and fungi in acid mine drainage environment with special reference to metals and sulfate immobilization. Water Res 43:883–894.

de-Bashan LE, Bashan Y (2010) Immobilized microalgae for removing pollutants: Review of practical aspects. Bioresour Technol 101:1611–1627.

De-Luca R, Bezzo F, Béchet Q, Bernard O (2017) Exploiting meteorological forecasts for the optimal operation of algal ponds. J Process Control 55:55–65.

de Wilt A, Butkovskyi A, Tuantet K, Leal LH, Fernanes TV, Langenhoff A, Zeeman G (2016) Micropollutant removal in an algal treatment system fed with source separated wastewater streams. J Hazard Mater 304:84–92.

Delrue F, Álvarez-Díaz PD, Fon-Sing S, Fleury G, Sassi J-F (2016) The environmental biorefinery: Using microalgae to remediate wastewater, a win-win paradigm. Energies 9:1–19. doi: 10.3390/en9030132

Dong B, Ho N, Ogden KL, Arnold RG (2014) Cultivation of Nannochloropsis salina in municipal wastewater or digester centrate. Ecotoxicol Environ Saf 103:45–53.

Duong VT, Li Y, Nowak E, Schenk PM (2012) Microalgae isolation and selection for prospective biodiesel production. Energies 5:1835–1849.

Dwivedi S (2012) Bioremediation of heavy metal by algae: Current and future perspective. J Adv Lab Res Biol 3:195–199.

Ekendahl S, Bark M, Engelbrektsson J, Karlsson C-A, Niyitegeka D, Strömberg N (2018) Energy-efficient outdoor cultivation of oleaginous microalgae at northern latitudes using waste heat and flue gas from a pulp and paper mill. Algal Res 31:138–146.

Environmental Protection Agency (1997) Wastewater Treatment Manuals: primary, secondary and tertiary treatment. Ardcavan, Wexford, Ireland.

Ferro L, Gentili FG, Funk C (2018) Isolation and characterization of microalgal strains for biomass production and wastewater reclamation in Northern Sweden. Algal Res 32:44–53.

Fukuda S-Y, Iwamoto K, Atsumi M, Yokoyama A, Nakayama T, Ishida K, Inouye I, Shiraiwa Y (2014) Global searches for microalgae and aquatic plants that can eliminate radioactive cesium, iodine and strontium from the radio-polluted aquatic environment: a bioremediation strategy. J Plant Res 127:79–89.

Gao F, Peng YY, Li C, Cui W, Yang W-H, Zeng G-M (2018) Coupled nutrient removal from secondary effluent and algal biomass production in membrane photobioreactor (MPBR): Effect of HRT and long-term operation. Chem Eng J 335:169–175.

Ge S, Qiu S, Tremblay D, Viner K, Champagne P, Jessop P (2018) Centrate wastewater treatment with Chlorella vulgaris: Simultaneous enhancement of nutrient removal, biomass and lipid production. Chem Eng J 342:310–320.

Gómez-Pérez CA, Espinosa J, Montenegro-Ruiz LC, van Boxtel AJB (2015) CFD simulation for reduced energy costs in tubular photobioreactors using wall turbulence promoters. Algal Res 12:1–9. doi: 10.1016/j.algal.2015.07.011

Gómez-Pérez CA, Espinosa-Oviedo JJ, Montenegro-Ruiz LC, van Boxtel AJB (2017) Twisted tubular photobioreactor fluid dynamics evaluation for energy consumption minimization. Algal Res 27:65–72. doi: 10.1016/j.algal.2017.08.019

Gordon R, Seckbach J (eds) (2012) The Science of Algal Fuels. Springer, Dordrecht. p 517

Gouveia L, Graça S, Sousa C, Ambrosano L, Ribeiro B, Botrel EP, Castro-Neto P, Ferreira AF, Silva CM (2016) Microalgae biomass production using wastewater: Treatment and costs. Scale-up considerations. Algal Res 16:167–176.

Guldhe A, Kumari S, Ramanna L, Ramsundar P, Singh P, Rawat I, Bux F (2017) Prospects, recent advancements and challenges of different wastewater streams for microalgal cultivation. J Environ Manage 203:299–315.

Gutiérrez-Alfaro S, Rueda-Márquez JJ, Perales JA, Manzano MA (2018) Combining sun-based technologies (microalgae and solar disinfection) for urban wastewater regeneration. Sci Total Environ 619–620:1049–1057.

Gutiérrez R, Ferrer I, Uggetti E, Arnabat C, Salvadó H, García J (2016) Settling velocity distribution of microalgal biomass from urban wastewater treatment high rate algal ponds. Algal Res 16:409–417.

Hamawand I, Yusaf T, Hamawand S (2014) Growing algae using water from coal seam gas industry and harvesting using an innovative technique: A review and a potential. Fuel 117:422–430.

Harris L, Tozzi S, Wiley P, Young C, Richardson T-MJ Clark K, Trent JD (2013) Potential impact of biofouling on the photobioreactors of the Offshore Membrane Enclosures for Growing Algae (OMEGA) system. Bioresour Technol 144:420–428.

Healy MG, Fenton O, Forrestal PJ, Danaher M, Brennan RB, Morrison L (2016) Metal concentrations in lime stabilised, thermally dried and anaerobically digested sewage sludges. Waste Manag 48:404–408.

Hecky R, Kilham P (1988) Nutrient limitation of phytoplankton in freshwater and marine environments : A review of recent evidence on the effects of enrichment. Limnol Ocenography 33:796–822.

Henkanatte-Gedera SM, Selvaratnam T, Karbakhshravari M, Myint M, Nirmalakhandan N, Voorhies WV, Lammers PJ (2017) Removal of dissolved organic carbon and nutrients from urban wastewaters by Galdieria sulphuraria: Laboratory to field scale demonstration. Algal Res 24:450–456.

Hii Y, Soo C, Chuah T (2011) Interactive effect of ammonia and nitrate on the nitrogen uptake by Nannochloropsis sp. J Sustain Sci Manag 6:60–68.

Hu Z, Qi Y, Zhao L, Chen G (2018) Interactions between microalgae and microorganisms for wastewater remediation and biofuel production. Waste Biomass Valori 0:1–13. doi: 10.1007/s12649-018-0325-7

Hughes AR, Sulesky A, Andersson B, Peers G (2018) Sulfate amendment improves the growth and bioremediation capacity of a cyanobacteria cultured on municipal wastewater centrate. Algal Res 32:30–37.

Hunter P, MacDonald A, Carter R (2010) Water supply and health. PLoS Med 7:e1000361. doi: 10.1371/journal.pmed.1000361

Kim CW, Sung MG, Nam K, Moon M, Kwon J-H, Yang J-W (2014) Effect of monochromatic illumination on lipid accumulation of Nannochloropsis gaditana under continuous cultivation. Bioresour Technol 159:30–35.

Luo Y, Le-clech P, Henderson RK (2017) Simultaneous microalgae cultivation and wastewater treatment in submerged membrane photobioreactors : A review. Algal Res 24:425–437.

Mackey KRM, Rivlin T, Grossman AR, Post AF, Paytan A (2009) Picophytoplankton responses to changing nutrient and light regimes during a bloom. Mar Biol 156:1531–1546.

Maeng SK, Khan W, Park JW, Han I, Yang HS, Song KG, Choi WJ, Kim S, Kim S, Woo H, Kim H-C (2018a) Treatment of highly saline RO concentrate using Scenedesmus quadricauda for enhanced removal of refractory organic matter. Desalination 430:128–135.

Maeng SK, You SH, Nam JY, Ryu H, Timmes TC, Kim HC (2018b) The growth of Scenedesmus quadricauda in RO concentrate and the impacts on refractory organic matter, Escherichia coli, and trace organic compounds. Water Res 134:292–300.

Makareviciene V, Skorupskaite V, Andruleviciute V (2013) Biodiesel fuel from microalgae-promising alternative fuel for the future: a review. Rev Environ Sci Biotechnol 12:119–130.

Matamoros V, Gutiérrez R, Ferrer I, García J, Bayona JM (2015) Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study. J Hazard Mater 288:34–42.

Maurer M, Boller M (1999) Modelling of phosphorus precipitation in wastewter treatment plants with enhanced biological phosphorus removal. Water Sci Technol 39:147–163.

Montemezzani V, Duggan IC, Hogg ID, Craggs RJ (2017a) Control of zooplankton populations in a wastewater treatment High Rate Algal Pond using overnight CO2 asphyxiation. Algal Res 26:250–264.

Montemezzani V, Duggan IC, Hogg ID, Craggs RJ (2017b) Assessment of potential zooplankton control treatments for wastewater treatment High Rate Algal Ponds. Algal Res 24:40–63.

Murwanashyaka T, Shen L, Ndayambaje JD, Wang Y, He N, Lu Y (2017) Kinetic and transcriptional exploration of Chlorella sorokiniana in heterotrophic cultivation for nutrients removal from wastewaters. Algal Res 24:467–476.

Novoveská L, Zapata AKM, Zabolotney JB, Atwood MC, Sundstrom ER (2016) Optimizing microalgae cultivation and wastewater treatment in large-scale offshore photobioreactors. Algal Res 18:86–94.

Noyola A, Morgan-Sagastume JM, Güereca LP (2013) Selección de tecnologías para el tratamiento de aguas residuales municipales. Universidad Nacional Aotónoma de México, México.

Olguín EJ (2012) Dual purpose microalgae-bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a Biorefinery. Biotechnol. Adv. 30:1031–1046.

Onda K, Lobuglio J, Bartram J (2012) Global access to safe water: Accounting for water quality and the resulting impact on MDG progress. Int J Environ Res Public Health 9:880–894.

Osundeko O, Pittman JK (2014) Implications of sludge liquor addition for wastewater-based open pond cultivation of microalgae for biofuel generation and pollutant remediation. Bioresour Technol 152:355–363.

Paredes D, Kuschk P, Mbwette TSA, Stange F, Müller RA, Köser H (2007) New aspects of microbial nitrogen transformations in the context of wastewater treatment - A review. Eng Life Sci 7:13–25.

Podola B, Li T, Melkonian M (2017) Porous substrate bioreactors: A paradigm shift in microalgal biotechnology? Trends Biotechnol 35:121–132.

Potera C (2011) Desechos peligrosos. Algas de los estanques aíslan el estroncio 90. Salud Publica Mex 53:363–364.

Rabalais NN, Turner RE, Díaz RJ, Justić D (2009) Global change and eutrophication of coastal waters. ICES J Mar Sci 66:1528–1537.

Razzak SA, Hossain MM, Lucky RA, Bassi AS, Lasa H (2013) Integrated CO2 capture, wastewater treatment and biofuel production by microalgae culturing—A review. Renew Sustain Energy Rev 27:622–653.

Romero-Lopez J, Lopez-Rodas V, Costas E (2012) Estimating the capability of microalgae to physiological acclimatization and genetic adaptation to petroleum and diesel oil contamination. Aquat Toxicol 124-125:227-237.

Sala L, Mujeriego R (2001) Cultural eutrophication control through water reuse. Water Sci Technol 43:109–116.

Salas-Herrera G, Benavides-Mendoza A, Zermeño-González A, Orta Dávila A, Sánchez-Pérez FJ (2015) Evaluación de microalgas para la producción de biomasa económicamente útil usando aguas producidas. Rev Mex Cienc Agric 12:2423-2435.

Sasongko NA, Noguchi R, Ito J, Demura M, Ichikawa S, Nakajima M, Watanabe MM (2018) Engineering study of a pilot scale process plant for microalgae-oil production utilizing municipal wastewater and flue gases: Fukushima pilot plant. Energies 11,1693. doi: 10.3390/en11071693

Schultze LKP, Simon MV, Li T, Langenbach D, Podola B, Melkonian M (2015) High light and carbon dioxide optimize surface productivity in a twin-layer biofilm photobioreactor. Algal Res 8:37–44.

Sforza E, Simionato D, Giacometti GM, Bertucco A, Morosinotto T (2012) Adjusted light and dark cycles can optimize photosynthetic efficiency in algae growing in photobioreactors. PLoS One e38975. doi: 10.1371/journal.pone.0038975

Shariati M, Hadi MR (2011) Microalgal biotechnology and bioenergy in Dunaliella. In: Carpi A (ed) Progress in molecular and environmental bioengineering- From analysis and modeling to technology applications. InTech, Shanghai, pp 447–506.

Sriram S, Seenivasan R (2012) Microalgae Cultivation in Wastewater for Nutrient Removal. J Algal Biomass Utln 3:9–13.

Sullivan EJ, Dean CA, Yoshida TM, Steichen SA, Laur PA, Visolay AW (2012) Feasibility and treatment of oil and gas produced water as a medium for Nannochloropsis salina cultivation. United States. https://www.osti.gov/servlets/purl/1043028.

Ting H, Haifeng L, Shanshan M, Zhang Y, Zhidan L, Na D (2017) Progress in microalgae cultivation photobioreactors and applications in wastewater treatment: A review. Int. J. Agric. Biol. Eng. 10:1–29. doi:10.3965/j.ijabe.20171001.2705

Tsai DD-W, Ramaraj R, Chen PH (2012) Growth condition study of algae function in ecosystem for CO2 bio-fixation. J Photoch Photobio B 107:27–34.

Wahidin S, Idris A, Shaleh SRM (2013) The influence of light intensity and photoperiod on the growth and lipid content of microalgae Nannochloropsis sp. Bioresour Technol 129:7–11.

Wang J, Zhuang L, Xu X, Deantes-espinosa VM, Wang X-X, Hu H-Y (2018) Microalgal attachment and attached systems for biomass production and wastewater treatment. Renew Sustain Energy Rev 92:331–342.

Wang M, Chen Y (2018) Generation and characterization of DOM in wastewater treatment processes. Chemosphere 201:96–109.

Wenk J, Aeschbacher M, Sander M, Gunten U, Canonica S (2015) Photosensitizing and inhibitory effects of ozonated dissolved organic matter on triplet-induced contaminant transformation. Environ Sci Technol 49:8541–8549.

Xiong JQ, Kurade MB, Jeon BH (2018) Can Microalgae Remove Pharmaceutical Contaminants from Water? Trends Biotechnol 36:30–44.

Yun J, Cho D, Lee S, Heo H, Tran QG, Chang YK, Kim HS (2018) Hybrid operation of photobioreactor and wastewater-fed open raceway ponds enhances the dominance of target algal species and algal biomass production. Algal Res 29:319–329.

Zhang J, Su M, Xi B, Qian G, Liu J, Hua F, Huo S (2016) Algal uptake of dissolved organic nitrogen in wastewater treatment plants. J Environ Sci-China 50:56–64.

Figures

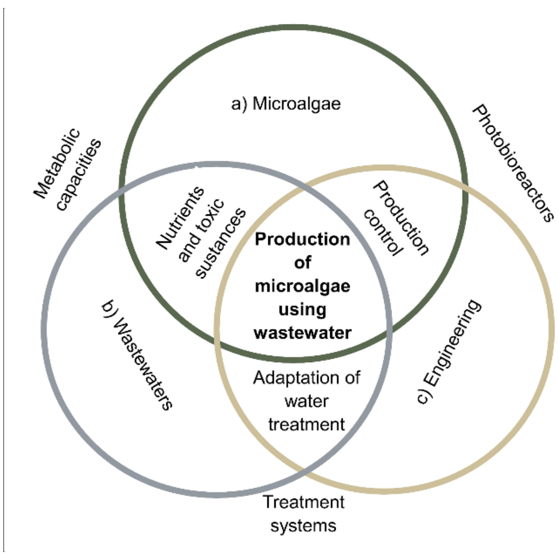

Fig 1. For the production of microalgae in wastewater should be considered the characteristics of water (the advantages and disadvantages that could represent for microalgae), and the adaptive capacities of the strains, in addition to the necessary engineering to favor the development of the culture, taking into account both the needs of microalgae and the challenges that could be faced when using wastewater.

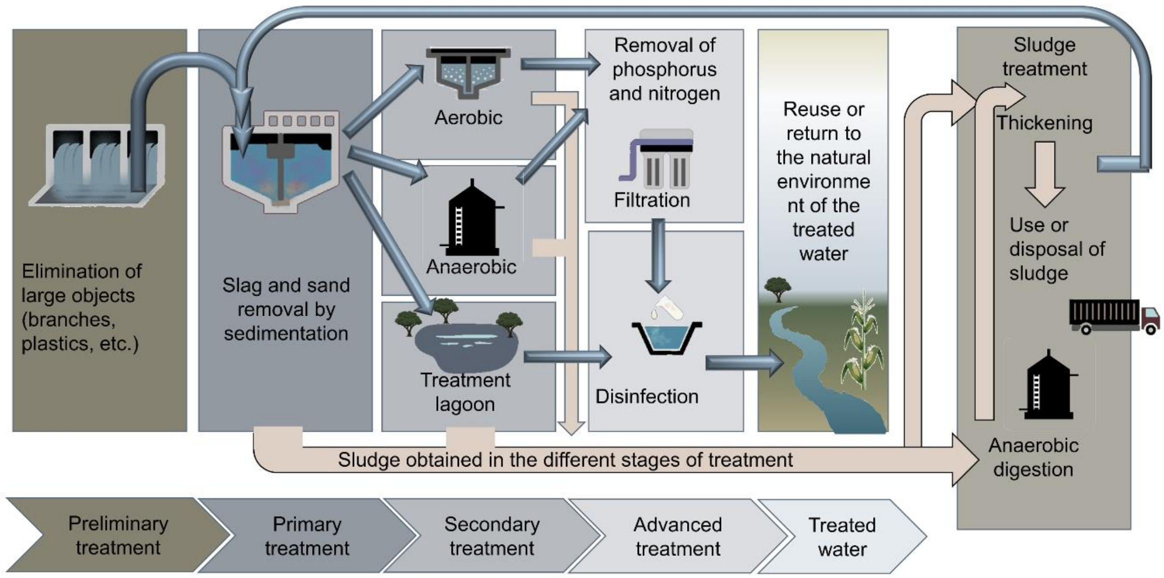

Fig 2. Generalized scheme of different processes and possible ways in the treatment of wastewater for the identification of the different effluents used for the exploitation with the cultivation of microalgae.

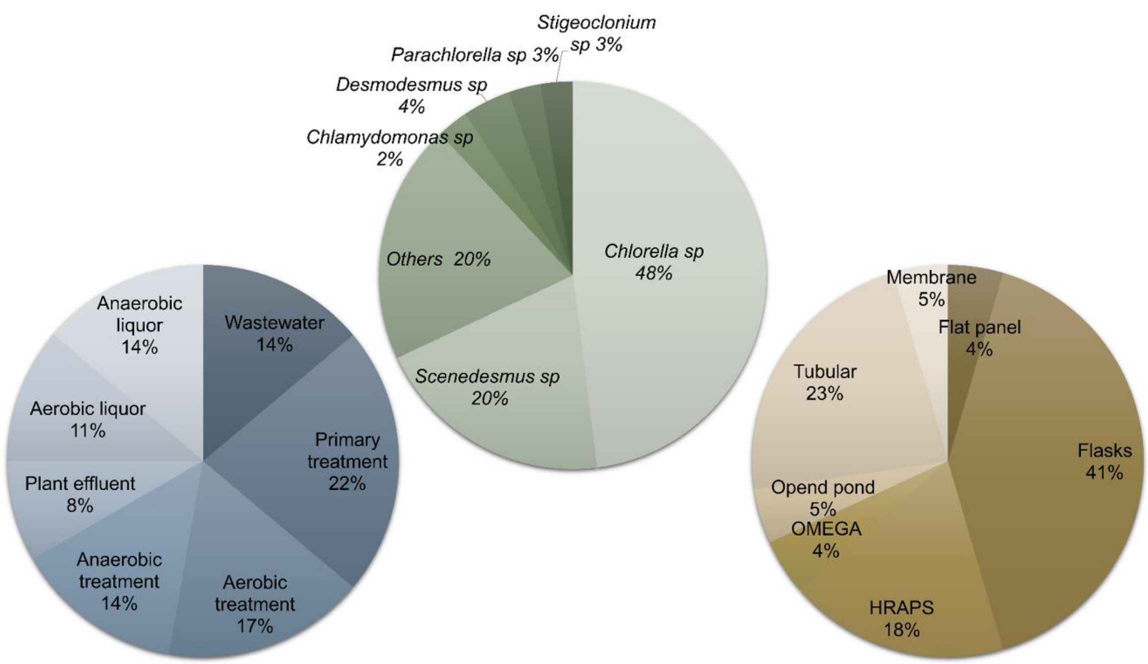

Fig 3. Compiled information of the research works reviewed in this document. The percentage refers to the mentions in the experiments, however, non-specific when combinations of wastewater effluents, combined cultures of strains or combined production systems were used. a) Effluent of treatment water used for experiments. b) Genus of strains evaluated. c) Type of system used.

CAPITULO DE LIBRO

Agronanobiotechnologies to Improve the Water Quality in Irrigation Systems

Chapter 8
Agronanobiotechnologies to Improve the Water Quality in Irrigation Systems

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Abstract Several international studies have shown that the performance of watering practices and irrigation equipment are still too low, while the water quality and availability are increasingly scarce worldwide. Consequently, there are reductions in crop yields and a waste of water resources. The objectives of this chapter are (1) discussing some bibliographic evidence regarding the availability of agronanobiotechnologies to improve the water quality and watering efficiency in agricultural irrigation systems and (2) describing some technological developments used in the design of cheap and eco-friendly filters with natural or engineering nanomaterials and organic wastes. It has been found that groundwater irrigation has grown rapidly

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over the past 50 years and now supplies over one-third of the world's irrigated area. Water management emerged as a strategic resource, not only in many arid and semi-arid countries, but also in humid climates, because of its capacity to support intensive land use and high-value agriculture. However, effective governance of watering water and the implementation cutting-edge technologies are critical and urgent challenges. It is required to critically examine the various approaches that different technologies have proposed for taking advantage sustainably about irrigation water and assessing their wider applicability for promoting its responsible use worldwide, while better water technologies and management are urgent and critical for productivity, equity, and sustainability.

Keywords Crop water requirements · Engineering nanomaterials · Irrigation and drainage · Rainfall harvesting · Runoff and evaporation · Low-cost irrigation techniques · Nanofilter · Water supply

1 Introduction

Water scarcity and the little availability of good quality water are global problems for human consumption or agricultural irrigated land. During the last years, high attention has started being paid on environmental analyses with multiple goals: quantifying environmental impacts of processes, identifying environmental hotspots, and suggesting mitigation strategies to reduce the impact of anthropogenic productions on the environment (Lovarelli et al. 2016).

Global consumption of freshwater resources has grown more than sixfold in the past century, and local water consumption has accumulated as a global problem (Luan et al. 2018). In addition, human impact on the environment has grown much more and faster than what was expected, and humanity consumes more resources (e.g., land, water) than what Earth is capable of regenerating (Galli et al. 2012).

Nowadays water scarcity is a major issue for present and future generations. It is well known that globally less than 10% of collected wastewater receives any form of treatment. Concomitantly, agriculture is the largest water user in most countries, representing 70% of total global freshwater withdrawals (Thebo et al. 2017). However, drought and inadequate water management are the predominant causes of low yields worldwide so that there is an urgent need for more water-efficient cropping systems facing large water consumption of irrigated agriculture and high unproductive losses via runoff and evaporation. Consequently, identification of yield-limiting constraints in the plant-soil-atmosphere continuum is the key to improved management of plant water stress (Bodner et al. 2015). Nevertheless, it has to be remembered that other strategies such as deficit irrigation have been widely investigated as a valuable and sustainable production strategy in dry regions, while limiting water applications to drought-sensitive growth stages aims to maximize water productivity and to stabilize—rather than maximize—yields (Geerts and Raes 2009).

Despite technological efforts by the specialists from different knowledge areas, water scarcity, water pollution, runoff, and evaporation are main problems which link to the use of water in agricultural systems. In addition, water is becoming scarce not only in arid or drought-prone areas but also in regions where rainfall is abundant: water scarcity concerns the quality of resource available and the quality of the water because degraded water resources become unavailable for more requirements (Pereira et al. 2002). Pereira et al. (2002) also stated that the sustainable use of water (resource conservation, environmental friendliness, appropriateness of technologies, economic viability, and social acceptability of developments issues) is a priority for agriculture in water-scarce regions. Imbalances between availability and demand, degradation of surface and groundwater quality, inter-sectorial competition, and interregional and international conflicts often occur in water-shortage regions. Therefore, innovations are required mainly relative to irrigation management and practice since the agriculture sector is far ahead in demand for water in those regions.

The objectives of this chapter are (1) discussing some bibliographic evidence regarding the availability of agronanobiotechnologies to improve the water quality and watering efficiency in agricultural irrigation systems and (2) describing some technological developments used in the design of cheap and eco-friendly filters with natural or engineering nanomaterials and organic wastes.

2 Irrigation Versus Rain-Fed Agriculture

There are two main ways to use agricultural water to cultivate crops: (1) rain-fed farming and (2) irrigation. Rain-fed farming is the natural application of water to the soil through direct rainfall. Rainfall reduces the contamination of food products but is open to water shortages when rainfall is scarce. On the other hand, artificial applications of water increase the risk of contamination by heavy metals, organic or inorganic pollutants, or pathogen microorganisms (Table 8.1; Fernández-Luqueño et al. 2013). Irrigation is the artificial application of water to the crops through systems of tubes, pumps, and sprays. There are many types of irrigation systems, in which water is supplied to the entire field uniformly.

Irrigation water can come from groundwater, surface water, or even other sources, such as treated wastewater or desalinated water. As a result, it is critical that farmers protect their agricultural water source to minimize the potential for contamination. It is well known that rainfall generally is uncontaminated and it could be stored throughout rainfall harvesting for later use or used without any previous treatment. However, frequently the water stored, treated, or extracted for irrigation purposes requires several treatments to decrease the pollutants, salts, or pathogens, so that several novel materials with specific characteristics never seen before (Table 8.2) have been synthesized by nanotechnologies and they could be used to improve the irrigation water quality (Fig. 8.1).

Table 8.1 Main characteristics that affect the quality of agricultural irrigation water

Pollutant	Problems	Reference
Salinity	Salts in soil or water reduce water availability to the crop and cause a slow rate of growth, along with a suite of metabolic changes caused by water stress, including premature senescence	Munns (2002)
Ion toxicity	Sodium, chloride, and boron ions from soil or water accumulate in a sensitive crop to concentrations high enough to cause crop damage and reduce yields. It is usually first evidenced by marginal leaf burn and interveinal chlorosis	WHO (2006)
Pathogens	Diseases such as diarrhea, cholera, hepatitis A, and typhoid fever can be transmitted through direct physical contact of farmers with wastewater or by consumption of products irrigated with contaminated ground or water	Minhas et al. (2006) Hanjra et al. (2012)
Nutrients	High nitrogen concentrations in the water which supplies the crop may cause undesirable vegetative growth, delayed crop maturity, and reduced crop quality	Qadir et al. (2010)
Suspended solids	Organic and inorganic sediments cause problems in irrigation systems through clogging of gates, sprinkler heads, and drippers. Sediments also reduce water infiltration rate of an already slowly permeable soil	WHO (2006)
Heavy metals	Heavy metals accumulated in the edible parts of leafy vegetables. Consumption of heavy metal-contaminated food can cause a decrease in immunological defenses, intrauterine growth retardation, impaired psychosocial behavior, disabilities associated with malnutrition, and a high prevalence of upper gastrointestinal cancer	Arora et al. (2008)

3 Types of Irrigation Systems

There are many different types of irrigation systems, depending on how the water is distributed throughout the field. In addition, some modern technologies to watering in cropped soils are described in Table 8.3, while some common types of irrigation systems include:

1. Surface irrigation: water is distributed over and across land by gravity, no mechanical pump involved.
2. Localized irrigation: water is distributed under low pressure, through a piped network and applied to each plant.
3. Drip irrigation: localized irrigation in which drops of water are delivered at or close the root of plants.
4. Sprinkler irrigation: water is distributed by overhead high-pressure sprinklers or guns from a central location in the field or from sprinklers on moving platforms.
5. Center-pivot irrigation: water is distributed by a system of sprinklers that move on wheeled towers in a circular pattern. This system is common in flat areas.

Table 8.2 Properties of the main nanomaterials (NM) used for wastewater treatment

NM	Application	Mechanism of action	Reference
Cu	The use of copper nanoparticles in paper filters for water purification contaminated with bacterial activity	The CuNP papers with higher copper content showed a high bacteria reduction of for *Escherichia coli*	Dankovich and Smith (2014)
TiO_2	Textile-wasted water contaminated with methylene blue	TiO_2 nanoparticles degraded methylene blue from the solution due to the high photocatalytic activity	Hossain and Hossain (2015)
CuO	To purify seawater contaminated with oil	The use of CuO demonstrates that it could find promising application in oil-water separation and offshore oil spill cleanup	Kong et al. (2015)
Magnetic nano-adsorbent	Wastewater contaminated with Pb^{2+}	It improves 80% removal efficiency	Khani et al. (2016)
Fe_3O_4 and γ-Fe_2O_3	Wastewater contaminated with mercury	It removes mercury by 70%	Vélez et al. (2016)
Zeolite materials obtained from fly ash	Wastewater contaminated with Pb^{2+}	Improves >80% removal efficiency	Visa (2016)
TiO_2/CuO nanoneedle arrays (NNA)	Industrial water contaminated with oil	The nanostructure TiO_2/CuO NNA dual-coated meshes are potentially useful in practical oil/water separation	Yuan et al. (2017)

6. Lateral move irrigation: water is distributed through a series of pipes, each with a wheel and a set of sprinklers, which are rotated either by the hand or with a purpose-built mechanism.
7. Subirrigation: water is distributed across land by raising the water table, through a system of pumping stations, canals, gates, and ditches.
8. Manual irrigation: water is distributed across land through manual labor and watering cans.

4 The Value of Irrigation

Irrigation systems allow primary producers to grow more crops and to have more flexibility in their productive processes as the ability to access water at times when it would otherwise be hard to achieve good plant growth due to a deficit in soil moisture. Producers can then achieve higher yields and meet market demands especially if rainfall events do not occur to produce higher-quality crops as water stress can dramatically impact on the quality of farm produce to lengthen the growing

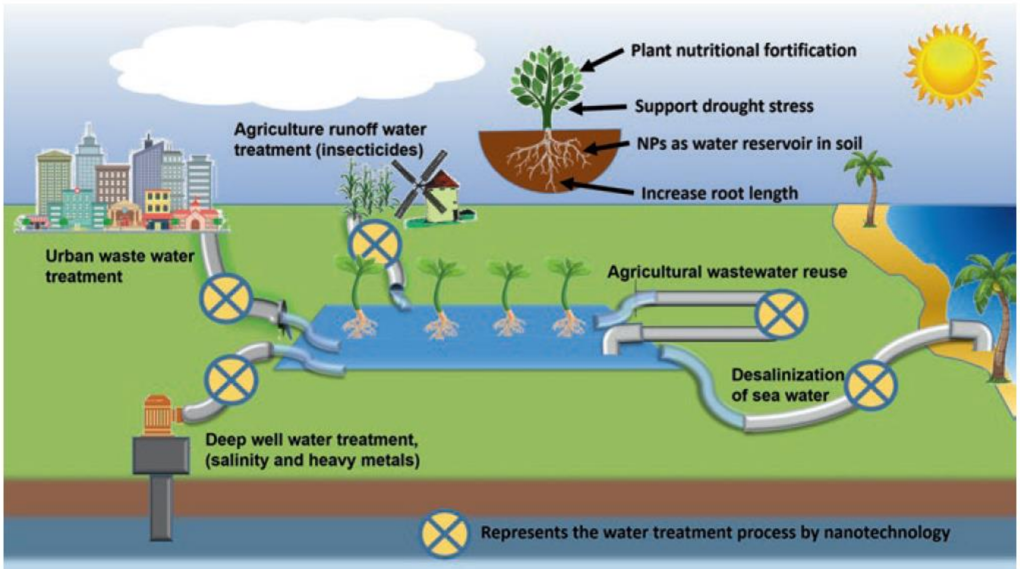

Fig. 8.1 Some of the possibilities where nanotechnology has been involved in irrigation water. Different sources of water, such as urban wastewater, salt water, deep well-contaminated water, or agricultural runoff waters, could be treated by nanotechnology to be used in agriculture. This nanotechnology includes nanomembranes and metal-based, carbon-based, or polymeric nanoadsorbents. Some of the contaminants which could be handled by nanotechnology are heavy metals, hydrocarbons, organic pollutants, and insecticides. Apart from water treatment, some nanomaterials applied by irrigation could have a positive impact in plant development or their quality. Examples are the increase in root area and length by Ag-NP, increased support to drought stress by maghemite nanoparticles, and fortification of plants for human consumption by Se-NP, while during drought conditions, calcium pectinate NP could act as a water reservoir

season to have "insurance" against seasonal variability and drought. Irrigation systems, wastewater management, and water store systems (Fig. 8.2) in cropped lands have several technical and financial benefits such as:

1. To stock more animals per hectare and practice tighter grazing management due to the reliability of pasture supply throughout the season.
2. To maximize benefits of fertilizer applications. Fertilizers need to be "watered into" the ground in order to best facilitate plant growth.
3. To use areas that would otherwise be "less productive." Irrigation can allow farmers to open up areas of their farms where it would otherwise be "too dry" to grow pasture/crops. This also gives them the capability to carry more stock or to conserve more feed.
4. To take advantage of market incentives for unseasonal production.
5. To have less reliance on supplementary feeding (grain, hay) in grazing operations due to the more consistent supply and quality of pastures grown under irrigation.
6. To improve the capital value of their property. Since irrigated land can potentially support higher crops, pasture, and animal production, it is considered more valuable. The value of the property is also related to the water licensing agreements or "water right."

Table 8.3 Modern systems for irrigation in agricultural production

Main argues and findings	Reference
Precision irrigation strategies, including variable rate irrigation, are useful approach for irrigation management to save water and reduce deep percolation losses.	Gonzáles-Perea et al. (2018)
Aerial sensor, with multispectral and infrared thermal imaging sensors, is a potential tool for remote crop stress monitoring. Green normalized vegetation index, canopy cover, and canopy temperature were able to differentiate crops with full and deficit irrigation at different growth stages.	Zhou et al. (2018)
Subsurface drip irrigation in rice cultivation produces similar grain yield compared with puddle-transplanted rice, with 50% lower N applications and 32% of water savings.	Rajwade et al. (2018)
Aquaponics is an integrated fish and plant production in a recirculation system. It has a hydroponic component which directly influences the water quality and consumption. The plant species influenced the daily water loss, whereas no effect was exerted by the water flow or type of hydroponics.	Maucieri et al. (2018)
Plant factories use the hydroponic techniques which have been used to increase the efficiency of protected horticulture. The hydroponic systems adopted in plant factories can circulate water and fertilizers within the systems.	Kikuchi et al. (2018)
Drip irrigation could reduce water consumption to 70% compared with conventional flood irrigation. Pressure compensate drip emitters to maintain a constant flow rate under variations in pressure have been designed and optimized empirically. A model to design new drip emitters with attributes that improve performance and lower cost is presented.	Shamshery et al. (2017)
Aeroponic system is a soilless culture system, where roots are kept in a dark environment saturated with aerosol of nutrient solution. Potato minituber production with this system resulted in a two to three times greater compared with the traditional method.	Rykaczewska (2016)
With the nutrient film technique, plants are grown directly in a circulated thin film of water containing a dissolved nutrient solution. This technique is easy to manipulate for toxicity test. The use of biochar filters reduced the Ni uptake in tomato plant growth with this technique.	Mosa et al. (2016)
Water productivity is increased by reducing non-beneficial use or by other agronomical practices such as engineering solutions that reduce the use of irrigation water. Agronomical solutions such as regulated deficit irrigation are directly linked to basin water conservation with little or no yield penalty.	Mateos and Araus (2016)

7. To cost save/obtain greater returns. The cost benefits from the more effective use of fertilizers and greater financial benefits as a result of more effective agricultural productivity (both quality and quantity) and for "out-of-season" production are likely.

5 Irrigation and Environment

Drainage facilities, in delta areas in particular, are considered as a form of flood protection. In conjunction with irrigation, they also prevent waterlogging and salinization. The area salinized by irrigation covers over 37 million ha worldwide, thereby reducing productivity. The use of urban wastewater in agriculture is a

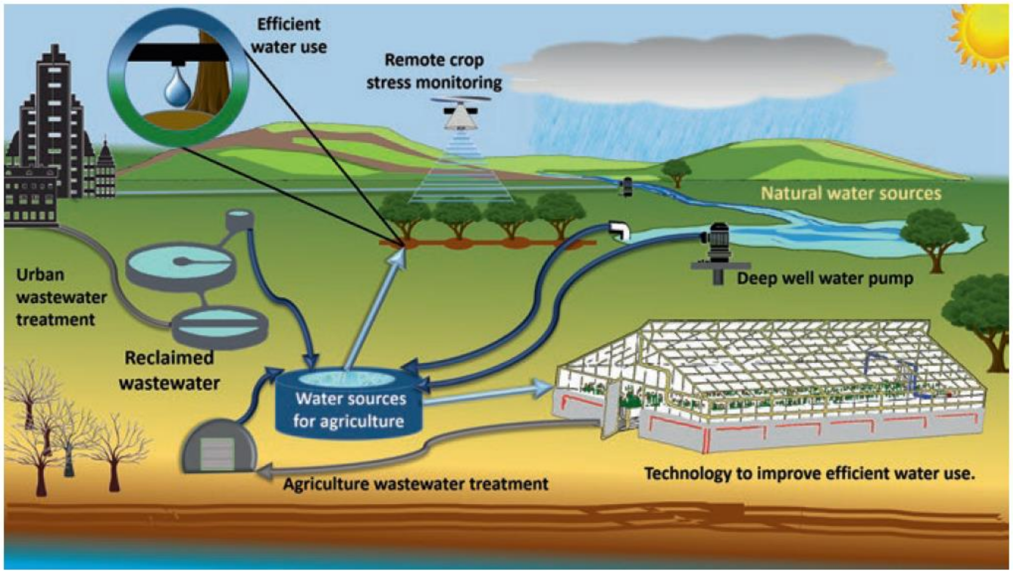

Fig. 8.2 Irrigation systems, wastewater management, and water store systems could improve the human well-being. To deal with food production and water scarcity, new technologies are emerging in water treatment and agricultural recirculation systems, as well as efficient water use. Reclaimed water from urban areas or agronomical activities could be a good water source together with natural water resources. Regulated deficit irrigation supported with remote crop stress monitoring is a promising emerging technology, while micro-irrigation systems in the field allow to give the amount of water that a plant needs by dripping water directly to the root zone. In protected agriculture, hydroponics and their variables increase the water use efficiency in crops and allow to recover the excess of water to be treated and recirculated into the production system. These are some of the actual approaches and future perspectives to deal with water scarcity and food production

century-old practice that is receiving renewed attention with the increasing shortage of freshwater around the world.

Irrigation of crops with wastewater is a common practice in urban and suburban farming communities where wastewater is often the only water source for agriculture. Additionally, wastewater contains important nutrients, such as inorganic N, P, micronutrients, and organic matter, which favor crop growth, but irrigating crops with wastewater might increase human viral and bacterial infections and contamination of the environment with toxic substances. In Latin America more than 500,000 ha arable land is irrigated with wastewater, of which 350,000 ha in México. In the valley of the Mezquital in the state of Hidalgo (México), 145,000 ha are irrigated with wastewater from Mexico City. This has favored the development of the region, but 1,200 ha have already been lost as agricultural land due to increased soil salt contents (Fernández-Luqueño et al. 2010).

Overexploitation of groundwater when water withdrawal exceeds water recharge—and its subsequent lowering of water tables—is a recurring problem in several cropping lands. In some countries the overpumping due to subsidizing electricity has lowered the water level by 25–30 m in one decade.

Fortunately, almost 155 million ha are under conservation agriculture worldwide. This technique enhances water use efficiency in rain-fed conditions due to minimum soil disturbance, soil cover, and appropriate crop association.

Some wetlands and inland valley bottoms are cultivated with minimum disturbance to the environment, as they have no or limited (mostly traditional) equipment to regulate water and control drainage. In addition, flood recession cropping is another traditional water management technique with relatively low environmental impact, where cultivation occurs along rivers in the areas exposed as floods recede and where nothing is undertaken to retain the receding water. It is well known that over 8.6 million ha worldwide are cultivated with these water managements.

However, there are also some examples of environmental problems regarding improper irrigation system management such as the drying up of the Aral Sea in Central Asia. It is one of the most dramatic examples of environmental tragedy caused by the mismanagement of irrigation where the sea level dropped by 17 m and the shoreline moved 70 km since 1960. This is due to the large diversions of water for irrigation of cotton and electricity production, resulting in little water reaching the Aral Sea. However, on a positive side, without the high productivity permitted by irrigation, at least an additional 500 million ha would be needed to reach the current agricultural production.

Temperate or humid areas allowing rain-fed production are often already densely populated or environmentally disturbed, therefore having no additional land for agriculture available anymore. Unfortunately, countries reaching their limit of cultivated areas already buy or rent large areas in other less developed countries, also known as land grabbing, i.e., they destroy and buy more cropping soil but do not improve technologies to take care the environment; they only look for economic benefits. In addition, globally more than one-third of the food is lost between field and fork, and thus also a large amount of water and energy, needed to produce the food. While in poor countries, most losses occur due to postharvest losses, in rich countries losses are mainly due to throwing away the food that is not consumed.

More reclaimed water is expected to be used for agricultural irrigation as the conventional water supply is becoming increasingly limited. The increasing concern of environmental risk caused by irrigation with reclaimed water and its complexity requires continuous monitoring and more research on the negative influences resulting from reclaimed water irrigation. To face these problems, the cutting-edge knowledge has been ahead, and new agronanobiotechnologies and/or biotechnologies have been developed during the last years in order to increase the yields and quality of harmless food (Tables 8.4 and 8.5).

According to Wang et al. (2017), extensive research regarding the extensive use of reclaimed water has shown a positive effect of reclaimed water irrigation on crop growth and yield with acceptable product qualities, although a reduction in the crop yield and quality and the ornamental performance of landscapes, as well as soil deterioration, have been occasionally reported. At present, there are some issues of great concern that should be addressed for the sustainable use of reclaimed water irrigation such as (Wang et al. 2017) (1) updating of the standards of reclaimed

Table 8.4 Agronanobiotechnologies to improve the water quality in agriculture irrigation systems

Main argues and findings	Reference
To use municipal wastewater for irrigation, it needs to be treated with plant growth-promoting rhizobacteria (PGPR) and Ag-NPs prior to be used for irrigation. Silver nanoparticles though suppressing the growth-promoting potential of PGPR increases their bioremediation potential for Pb, Cd, and Ni. Ag-NPs enhanced root area and root length by PGPR isolates.	Khan and Bano (2016)
Selenium NP uptake by wheat seedlings is dependent on nanoparticle size and synthesis method in hydroponic experiments. The selenium NP uptake is energy independent.	Hu et al. (2018)
Calcium pectinate nanoparticles function as water reservoirs to provide sustained irrigation in areas where water is scarce.	Sharma et al. (2017)
Maghemite nanoparticles delivered by irrigation support drought stress management through enzymatic activity in *Brassica napus.*	Palmqvist et al. (2017)
Main applications of nanotechnology in water bioremediation are as uranium remediation, hydrocarbon remediation, groundwater and wastewater remediation, and heavy metal remediation.	Dasgupta et al. (2017)
A hybrid system of forward osmosis and nanofiltration (FO-NF) for agricultural wastewater reuse was developed. FO-NF permeate showed a high-quality water for irrigation in a long-term period.	Corzo et al. (2018)
Low-quality waters can be filtered using nanotechnology applications allowing the removal of salts and other micropollutants. This water could be used for agricultural production.	Bueno et al. (2017)
Nanohexagon NiO sheets can potentially remove hydrophilic and hydrophobic insecticides such as carbamates and organochlorines, respectively, from agriculture wastewater.	Derbalah et al. (2015)
Wastewater and desalination for a more sustainable agriculture could be accomplished by nanomaterials science.	Villaseñor and Ríos (2018)

Table 8.5 Applied biotechnology to improve irrigation water

Main argues and findings	Reference
Two *Pseudomonas protegens* strains were isolated from an agricultural water well contaminated with heavy metals. The isolates show mycelial growth inhibition against some pathogenic fungus and have a potential as beneficial bacteria for agriculture applications even in metal-polluted soils.	Bensidhoum et al. (2016)
This study highlights the potential benefits that plant growth-promoting microorganisms may confer to plants grown in hydroponic systems, particularly when cultivated in extreme environments.	Sheridan et al. (2017)
From 48 bacterial strains isolated from agricultural water well, 4 shows the ability to express plant growth-promoting traits and inhibition of mycelia growth to *Botrytis cinerea* and *Aspergillus niger.*	Tabli et al. (2018)
Microalgae *Chlorella* sp. in aquaponics system is able to remove ammonia and balance pH drop caused by nitrifying bacteria. Algae prefer ammonia nitrogen over nitrate nitrogen.	Addy et al. (2017)
Stevia rebaudiana showed increase production of stevioside when treated with purple phototropic bacteria. Foliar treatments combined with treatments through rhizosphere irrigation showed best results.	Wu et al. (2013)

water for irrigation, (2) better understanding of the mechanisms of the migration, transformation, accumulation, and diffusion of various contaminants, (3) determining the technical parameters of irrigation systems to enhance the safety and effectiveness of reclaimed water irrigation, (4) making a risk assessment for continuous reclaimed water irrigation, (5) promoting local and global policies for developing reclaimed water irrigation, and (6) developing and evaluating new technologies that guarantee better performance of irrigation techniques without jeopardizing the sustainable development.

6 Design and Manufacture of Low-Cost and Environmentally Friendly Filters

Our research team has been working with the synthesis and evaluation of new materials to increase the performance of environmentally friendly water filters.

6.1 Methodology

Aspergillus niger strain (ATCC 9642) was obtained from the National Collection of Microbial Strains and Cell Cultures of Cinvestav Zacatenco, Mexico. It was subcultivated every month in malt extract agar. A Tween 80 (20% v/v) sterile stock solution was used for the spore dispersal. 2.5 mL spore suspension of *A. niger* ATCC 9642, obtained from a 14-day agar growth, was inoculated into 250 mL malt extract broth medium in a 500 mL Erlenmeyer flask. The cultures were cultivated at 30 °C and pH 4 for 6 days in an orbital incubator. Mycelium was recovered through filtration using filter paper (Whatman No. 2), washed repeatedly with distilled water until a clear filtrate was acquired, and dried for 3 h at 80 °C. Fungal biomass was homogenized using an Agate mortar and deproteinized with 15 mL 1 M NaOH treatment for 2 h at 90 °C. The alkali insoluble fraction was recovered by centrifugation (15,000 × *g*, 15 min), washed with distilled water, and recentrifuged until it reached a neutral pH. Finally, fungal biomass was dried and ground with Agate mortar.

Montmorillonite clay was extracted from 20 kg of vertisol soil by the test tube method. 50 g of soil, previously sieved in a 30 mesh, were air dried and placed in a 1-L test tube. 10 mL of sodium hexametaphosphate (5 g per 100 mL) were added and after 5 min stirred, and the test tube was left to settle for 24 h.

6.2 Adsorption Experiments

The adsorption of arsenic (As), lead (Pb), carbonate calcium ($CaCO_3$), and sulfate (SO_4^{-2}) ions was evaluated using produced fungal biomass, montmorillonite clay, and TiO2, Fe_2O_3, ZnO nanoparticles as adsorbents in aqueous solution. Adsorption experiments were conducted in a batch mode as a function of time (0–480 min) and concentration at neutral pH and 25 °C. A known weight of adsorbent (0.1 g) was added to 25 mL of composite solution containing equimolar concentrations of each compound in the range of 1–50 mg L^{-1}. The residual ion content was determined by inductively coupled plasma, and the capacity of adsorption (Q_e) was calculated according to the following equation:

$$Q_e = \frac{(C_o - C_e) * V}{m}$$

where "C_o" is the initial concentration (mg/L), "C_e" the equilibrium concentration (mg L^{-1}), "*m*" the weight of used adsorbent (g), and "*V*" the volume of the solution (L).

6.3 Application of the Adsorbents in a Filter

A PVC cylinder (30 cm of height and 2.4 cm of diameter) was designed and loaded with the five adsorbents to be applied as a filter. Contaminated water with As, Pb, $CaCO_3$, and SO_4 ions was passed from the bottom of the filter and released from the top at a continuous flow. Experiments were conducted in triplicate, and samples were collected each 2 h. Efficiency of the filter was evaluated by determining the pollutant concentration in the water release (Fig. 8.3).

6.4 Characterization

The physicochemical characteristics of the five adsorbent materials (fungal biomass, montmorillonite clay, and TiO_2, Fe_2O_3, and ZnO nanoparticles) were evaluated by Fourier-transform infrared spectroscopy (FT-IR), X-ray diffraction, X-ray fluorescence, and scanning electron microscopy (SEM).

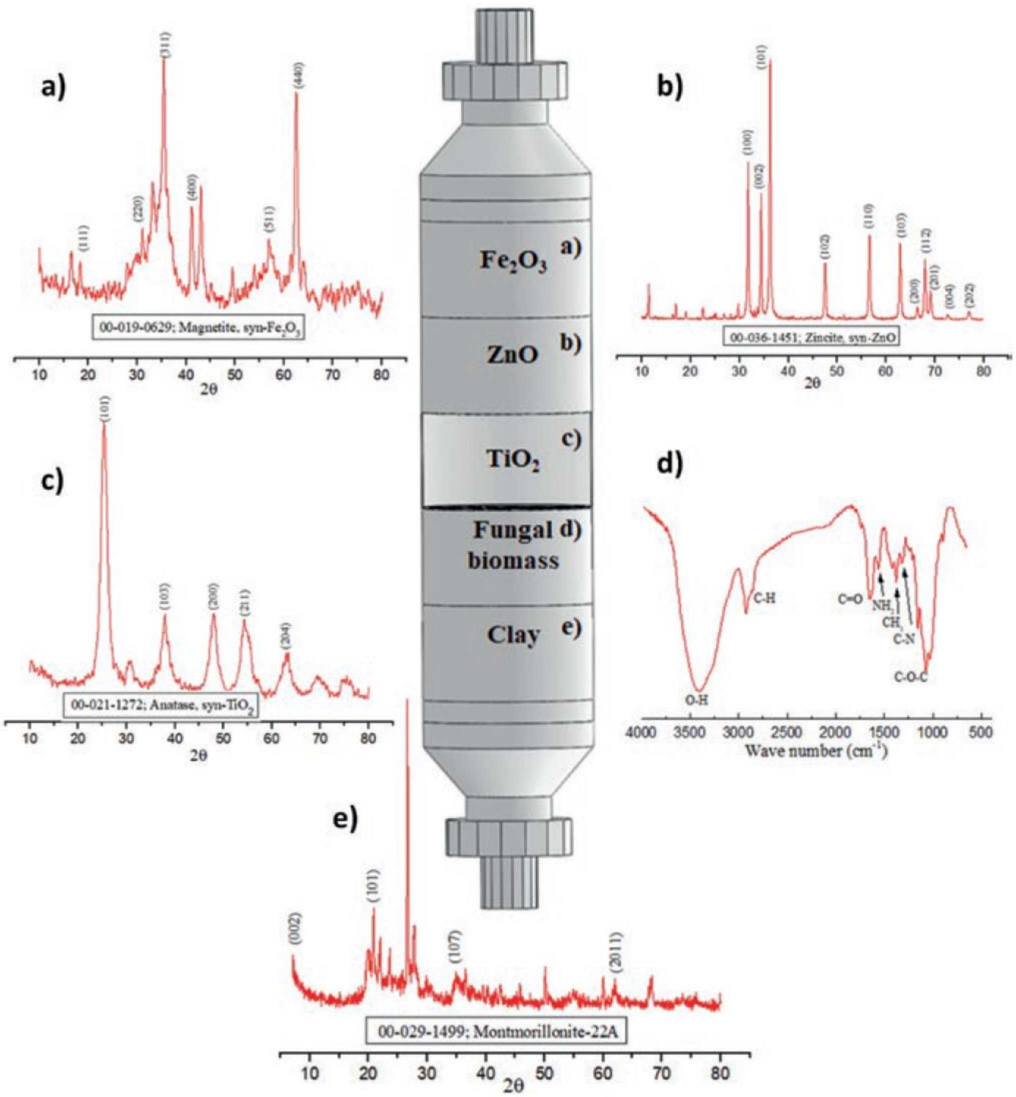

Fig. 8.3 Filter prototype with XRD diffractograms of (**a**) Fe_2O_3, magnetite, (**b**) ZnO, zincite and (**c**) TiO_2, anatase, (**d**) IR spectrum of fungal biomass, and (**e**) XRD diffractogram of montmorillonite clay

6.5 *Adsorption Yields*

Once the batch tests for each adsorbent have been carried out, it has been found that the adsorption equilibrium is reached after 2 h of contact between adsorbent and adsorbate. Regarding the removal efficiency of the pollutant mixture, taking into account an initial concentration of 25 mg L^{-1} of each one, a neutral pH, and a temperature of 25 °C, the lead ion has decreased by 89% and 98% using montmorillonite clay and the three nanoparticles, respectively. On the other hand, As concentration has been diminished by over 90% using the nanoparticles of ZnO (93%) and TiO_2 (98%), while the removal of $CaCO_3$ has only been favored with the nanoparticles of Fe_2O_3 in a 90%. The removal of the sulfate ion is very low, since none of the five adsorbents obtained a yield greater than 10%.

Our research team is also working on a low-cost solution for household water purification by a manufactured filter with engineering nanoparticles, soil-natural clays, and recycled materials. The goal of this research is to design, build, and evaluate a cheap water filter for the low-income household which is being manufactured with engineering nanoparticles (NP), natural soil NP, and recycled materials. In the present study, water filters were developed with Ag-NP, TiO_2-NP, coffee waste, and natural soil NP. Soil NP and residues of coffee-supported Ag-/TiO_2-NP (soil NP/coffee waste/Ag/TiO_2-NP) were prepared through step by step. First, the preparation of the coffee waste and the extraction of soil NP were made. After that, coffee residues and the soil NP were sifted, mixed, and dispersed in 25 mL of ethanol under continuous stirring until a suspension was formed. Then 0.2 g of $AgNO_3$ was dissolved in the suspension with stirring, followed by the addition of 1.5 mL of tetrabutyltitanate. After stirring for 2 h, the mixture was heated at 160 °C for 30 h, centrifuged, and calcined at 500 °C for 5 h to firmly attach among themselves. Powder X-ray diffraction (XRD) and transmission electron microscopy (TEM) showed that the Ag-NP coated with TiO_2-NP is well-dispersed on the surface of soil NP and recycled materials. This nanomaterial, i.e., soil NP/coffee waste/Ag/TiO_2-NP, had proper recycling, increased the surface area, and facilitated the water purification.

7 Conclusion

Suffering from severe water scarcity, several countries have been using wastewater for irrigating cereal, fiber, and vegetable crops. However, rarely both quantities and qualities have been enhanced from raw wastewater, i.e., the common procedure is to have irrigation system watering crops without any previous or minimum treatment.

The importance of promoting local and global policies for developing reclaimed water irrigation must be recognized worldwide. In addition, as integral parts of wastewater reclamation policy frameworks and its use and management in land watering systems, several regulations should be developed and improved worldwide.

Effective governance of watering and the implementation of cutting-edge technologies are critical and urgent challenges. It is required critically to examine the various approaches that different technologies have proposed for taking advantage sustainably about irrigation water and assessing their wider applicability for promoting its responsible use worldwide, while better watering technologies and management are urgent and critical for productivity, equity, and sustainability.

The synthesis of new materials for treated wastewater or freshwater with potential use in irrigation systems has to be promoted but also the long-term studies to know the potential human or environmental harm. The humanity needs more water, energy, and food, but also needs a comfortable and safe site to live and thrive. Otherwise, the sustainable development will be jeopardized.

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Conflict of interest The authors declare no conflict of interest.

References

Addy MM, Kabir F, Zhang R, Lu Q, Deng X, Current D, Griffith R, Ma Y, Zhou W, Chen P, Ruan R (2017) Co-cultivation of microalgae in aquaponics systems. Bioresour Technol 245:27–34

Arora M, Kiran B, Rani S, Rani A, Kaur B, Mittal N (2008) Heavy metal accumulation in vegetables irrigated with water from different sources. Food Chem 111(4):811–815

Bensidhoum L, Nabti E, Tabli N, Kupfershmied P, Weiss A, Rothballer M, Schmid M, Keel C, Hartmann A (2016) Heavy metal tolerant Pseudomonas protegens isolates from agricultural well water in northeastern Algeria with plant growth promoting, insecticidal and antifungal activities. Eur J Soil Biol 75:38–46

Bodner G, Nakhforoosh A, Kaul HP (2015) Management of crop water under drought: a review. Agron Sustain Dev 35(2):401–442

Bueno PD, Gillerman L, Gehr R, Oron G (2017) Nanotechnology for sustainable wastewater treatment and use for agricultural production: a comparative long-term study. Water Res 110:66–73

Corzo B, de la Torre T, Sans C, Escorihuela R, Navea S, Malfeito JJ (2018) Long-term evaluation of a forward osmosis-nanofiltration demonstration plant for wastewater reuse in agriculture. Chem Eng J 338:383–391

Dankovich TA, Smith JA (2014) Incorporation of copper nanoparticles into paper for point-of-use water purification. Water Res 63:245–251

Dasgupta N, Ranjan S, Ramalingam C (2017) Applications of nanotechnology in agriculture and water quality management. Environ Chem Lett 15(4):591–605

Derbalah A, El-Safty SA, Shenashen MA, Khairy M (2015) Hierarchical nanohexagon ceramic sheet layers as platform adsorbents for hydrophilic and hydrophobic insecticides from agricultural wastewater. ChemPlusChem 80(12):1769–1778

Fernández-Luqueño F, Reyes-Varela V, Cervantes-Santiago F, Gomez-Juarez C, Santillan-Arias A, Dendooven L (2010) Emissions of carbon dioxide, methane and nitrous oxide from soil receiving urban wastewater for maize (*Zea mays* L.) cultivation. Plant Soil 331(1–2):203–215

Fernández-Luqueño F, López-Valdez F, Gamero-Melo P, Luna-Suárez S, Aguilera-González EN, Martínez AI, García-Guillermo MDS, Hernández-Martínez G, Herrera-Mendoza R, Álvarez-Garza MA, Pérez-Velázquez IR (2013) Heavy metal pollution in drinking water—a global risk for human health: A review. J Environ Sci Technol 7(7):567–584

Galli A, Wiedmann T, Ercin E, Knoblauch D, Ewing B, Giljum S (2012) Integrating ecological, carbon and water footprint into a "Footprint Family" of indicators: definition and role in tracking human pressure on the planet. Ecol Indic 16:100–112

Geerts S, Raes D (2009) Deficit irrigation as an on-farm strategy to maximize crop water productivity in dry areas. Agric Water Manag 96(9):1275–1284

Gonzáles-Perea R, Daccache A, Rodríguez-Díaz JA, Camacho-Poyato E, Knox JM (2018) Modelling impacts of precision irrigation on crop yield and in-field management. Precis Agric 19:497–512

Hanjra MA, Blackwell J, Carr G, Zhang F, Jackson TM (2012) Wastewater irrigation and environmental health: Implications for water governance and public policy. Int J Hyg Environ Health 215(3):255–269

Hossain MF, Hossain MI (2015) Textile-wasted water cleaning by handmade screen printed TiO_2 nanoparticles. In: 2nd International Conference on Electrical Engineering and Information & Communication Technology (ICEEICT), Jahangirnagar University, Dhaka 1342, Bangladesh
Hu T, Li HF, Li JX, Zhao GS, Wu WL, Liu LP, Wang Q, Guo YB (2018) Absorption and biotransformation of selenium nanoparticles by wheat seedlings (*Triticum aestivum* L.). Front Plant Sci 9:597
Khan N, Bano A (2016) Role of plant growth promoting rhizobacteria and Ag-nano particle in the bioremediation of heavy metals and maize growth under municipal waste water irrigation. Int J Phytoremediation 18(3):211–221
Khani K, Sobhani S, Beyki MS (2016) Highly selective and efficient removal of lead with magnetic nano-adsorbent: multivariate optimization, isotherm and thermodynamic studies. J Colloid Interface Sci 466:198–205
Kikuchi Y, Kanematsu Y, Yoshikawa N, Okubo T, Takagaki M (2018) Environmental and resource use analysis of plant factories with energy technology options: a case study in Japan. J Clean Prod 186:703–717
Kong LH, Chen XH, Yu LG, Wu ZS, Zhang PY (2015) Superhydrophobic cuprous oxide nanostructures on phosphor-copper meshes and their oil–water separation and oil spill cleanup. ACS Appl Mater Interfaces 7:2616–2625
Lovarelli D, Bacenetti J, Fiala M (2016) Water Footprint of crop productions: A review. Sci Total Environ 548:236–251
Luan XB, Wu PT, Sun SK, Wang YB, Gao XR (2018) Quantitative study of the crop production water footprint using the SWAT model. Ecol Indic 89:1–10
Mateos L, Araus JL (2016) Hydrological, engineering, agronomical, breeding and physiological pathways for the effective and efficient use of water in agriculture. Agric Water Manag 164:190–196
Maucieri C, Nicoletto C, Junge R, Schmautz Z, Sambo P, Borin M (2018) Hydroponic systems and water management in aquaponics: a review. Ital J Agron 13(1):1–11
Minhas PS, Sharma N, Yadav RK, Joshi PK (2006) Prevalence and control of pathogenic contamination in some sewage irrigated vegetable, forage and cereal grain crops. Bioresour Technol 97(10):1174–1178
Mosa A, El-Banna MF, Gao B (2016) Biochar filters reduced the toxic effects of nickel on tomato (*Lycopersicon esculentum* L.) grown in nutrient film technique hydroponic system. Chemosphere 149:254–262
Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25(2):239–250
Palmqvist NGM, Seisenbaeva GA, Svedlindh P, Kessler VG (2017) Maghemite nanoparticles acts as nanozymes, improving growth and abiotic stress tolerance in *Brassica napus*. Nanoscale Res Lett 12:631
Pereira LS, Oweis T, Zairi A (2002) Irrigation management under water scarcity. Agric Water Manag 57(3):175–206
Qadir M, Wichelns D, Raschid-Sally L, McCornick PG, Drechsel P, Bahri A, Minhas PS (2010) The challenges of wastewater irrigation in developing countries. Agric Water Manag 97(4):561–568
Rajwade YA, Swain DK, Tiwari KN, Bhadoria PBS (2018) Grain yield, water productivity, and soil nitrogen dynamics in drip irrigated rice under varying nitrogen rates. Agron J 110(3):868–878
Rykaczewska K (2016) The potato minituber production from microtubers in aeroponic culture. Plant Soil Environ 62(5):210–214
Shamshery P, Wang RQ, Tran DV, Winter AG (2017) Modeling the future of irrigation: a parametric description of pressure compensating drip irrigation emitter performance. PLoS One 12(4):e0175241
Sharma R, Bajpai J, Bajpai AK, Somen A, Bhuvanesh K, Slingh RK (2017) Assessment of water retention performance of pectin-based nanocarriers for controlled irrigation in agriculture. Agric Res 6(2):139–149

Sheridan C, Depuydt P, De Ro M, Petit C, Van Gysegem E, Delaere P, Dixon M, Stasiak M, Aciksoz S, Frossard E, Paradiso R, De Pascale S, Ventorino V, De Meye T, Sas B, Geelen D (2017) Microbial community dynamics and response to plant growth-promoting microorganisms in the rhizosphere of four common food crops cultivated in hydroponics. Microb Ecol 73(2):378–393

Tabli N, Rai A, Bensidhoum L, Palmieri G, Gogliettino M, Cocca E, Consiglio C, Cillo F, Bubici G, Nabti E (2018) Plant growth promoting and inducible antifungal activities of irrigation well water-bacteria. Biol Control 117:78–86

Thebo AL, Drechsel P, Lambin EF, Nelson KL (2017) A global, spatially-explicit assessment of irrigated croplands influenced by urban wastewater flows. Environ Res Lett 12:074008

Vélez E, Campillo GE, Morales G, Hincapié C, Osorio J, Arnache O, Uribe JI, Jaramillo F (2016) Mercury removal in wastewater by iron oxide nanoparticles. J Phys Conf Ser 687:012050

Villaseñor MJ, Ríos A (2018) Nanomaterials for water cleaning and desalination, energy production, disinfection, agriculture and green chemistry. Environ Chem Lett 16(1):11–34

Visa M (2016) Synthesis and characterization of new zeolite materials obtained from fly ash for heavy metals removal in advanced wastewater treatment. Powder Technol 294:338–347

Wang Z, Li JS, Li YF (2017) Using reclaimed water for agricultural and landscape irrigation in China: a review. Irrig Drain 66(5):672–686

World Health Organization (2006) Guidelines for the safe use of wastewater, excreta and greywater, vol 1. WHO

Wu J, Wang Y, Lin X (2013) Purple phototrophic bacterium enhances stevioside yield by *Stevia rebaudiana* Bertoni via foliar spray and rhizosphere irrigation. PLoS One 8(6):e67644

Yuan S, Chen C, Raza A, Song R, Zhang TJ, Pehkonen SO, Liang B (2017) Nanostructured TiO_2/CuO dual-coated copper meshes with superhydrophilic, underwater superoleophobic and self-cleaning properties for highly efficient oil/water separation. Chem Eng J 328:497–510

Zhou J, Khot LR, Boydston RA, Miklas PN, Porter L (2018) Low altitude remote sensing technologies for crop stress monitoring: a case study on spatial and temporal monitoring of irrigated pinto bean. Precis Agric 19:555–569

CAPITULO DE LIBRO

Effects of Nanoparticles on Plants, Earthworms, and Microorganisms

Chapter 9
Effects of Nanoparticles on Plants, Earthworms, and Microorganisms

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Abstract The synthesis of engineered nanomaterials (ENMs) has increased in recent years because novel and unexpected properties and applications have been found to such a degree that hundreds of scientists have published concerns and evidence regarding the toxicology of ENMs. However, most of the reported findings have been inconsistent, so more research is needed, but also long-term in situ field trials are required, while the standardization of tests, chemical reagents, and methodologies must be strengthened and regulated in accordance with scientific advice or international organizations. This chapter discusses new findings published during

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the last 5 years regarding the advantages and disadvantages of ENMs, as well as findings obtained in our laboratories and greenhouse. We found that ENMs have favorable effects on some crops and biological systems. Consequently, ENMs have potential industrial applications in the agricultural sector, with biological, environmental, and ecological advantages. Nevertheless, the effects of ENMs depend on the kind of ENM, exposition period, concentration, substrate or soil type, kind and age of organisms, biotic and abiotic interactions, etc.; i.e., a specific test has to be carried out for each particular condition, and generalizations regarding the effects of ENMs should be avoided, otherwise human and environmental health—but also sustainable development—will be compromised.

Keywords Engineered nanomaterials · Human and environmental health · Sustainable development

1 Introduction

Nanomaterials are classified as naturally occurring, incidentally synthesized, and intentionally manufactured. Since engineered nanoparticles (ENPs) have been developed for use in industry and human commodities, it is common to find them in waste and by-products of industrial chemical reactions, but it is also possible to find incidental nanoparticles (NPs) in the environment (Medina-Pérez et al. in press). Despite that, nanotechnology has been recognized by the European Commission as one of its six “Key Enabling Technologies” that contribute to sustainable competitiveness and growth in several industrial sectors (Parisi et al. 2015).

According to Terekhova et al. (2017), ENPs can enter the soil through atmospheric precipitation, through sedimentation in the form of dust and aerosols, through direct soil absorption of gaseous compounds, through abscission of leaves, or as a result of anthropogenic activity, etc. After ENPs get into a water system through sewage or industrial emissions, nanoparticles can accumulate in plants (e.g., in algae), as well as in invertebrates (plankton, benthos, crustaceans) that are the primary links of a food chain, and then they can pass into water vertebrates that form part of the human food chain (Terekhova et al. 2017). In a land ecosystem, ENPs can accumulate in soil, vegetation, surface water, sewage, landfills, and groundwater.

The current challenges of sustainability, food security, and climate change are engaging researchers in exploring the field of nanotechnology as a new source of key improvements in the agricultural sector (Parisi et al. 2015). However, because of the rapid advent of nanotechnologies, great attention is being paid to the effects of engineered nanomaterials (ENMs) on living organisms, while concerns are rising in the scientific community worldwide.

Despite the numerous potential advantages of nanotechnology and the growing trends in publications and patents, agricultural applications have not yet made it to the market, but several factors could explain the scarcity of commercial applications, such as the high production costs of nanotechnological products, unclear technical benefits, and legislative uncertainties, as well as public opinion (Parisi et al. 2015). Nevertheless, the research and development landscape regarding ENMs is very promising, and the possibilities offered by nanoscience and nanotechnology in various agricultural applications will continue to be actively explored. In addition, the rapid progress of nanotechnology in other key industries may, over time, be transferred to agricultural applications as well, and facilitate their development (Parisi et al. 2015).

This chapter discusses new findings published during the last 5 years regarding the advantages and disadvantages of ENMs, as well as findings obtained in our laboratories and greenhouse.

2 Environmental Behavior of Engineered Nanomaterials at Various Trophic Levels

Despite the wide applications of ENPs in several areas, limited data are available on their behavior at various trophic levels. Rocha et al. (2017) stated that the current knowledge indicates the existence of important accumulation and ecotoxic effects of Cd-based quantum dots (QDs) on microorganisms, aquatic invertebrates, and vertebrates (fish) in freshwater and seawater.

It has to be acknowledged that there is an urgent need for development of analytical methods for detection and quantification of ENMs in environmental matrices, as well as a need to establish guidelines for experimental design and development of new end points/biomarkers for ecological risk assessment of ENMs. In addition, the ecotoxicology of ENMs in environmentally relevant exposure conditions, such as micro- and mesocosms, has not been investigated yet, while chronic and long-term ecotoxicity tests have been limited (Rocha et al. 2017). Entry, migration, transformation, or degradation of ENPs in different ecosystems (Fig. 9.1) have been reported by Cornelis et al. (2012), Keller and Lazareva (2014), Gokhale (2016), and Song et al. (2017).

According to Karimi et al. (2018), despite the wide application of nanoparticles in different sectors of the food industry and the benefits that nanotechnology offers in achieving better quality, safety, efficiency, and food-processing techniques, human exposure to nanoparticles through trophic transfer and possible adverse health effects on the human body seem to be inevitable. They also stated that toxicology of nanoparticles suffers from severe limitations in the certified assessment approaches and contradictions in the stated data, and that consideration should be given to screening of nanoparticle-containing foods in a set of long-term studies conducted in large groups of people to consider all of the related issues before those foods rapidly occupy the market.

Most nanoparticles have been traced in different plants, crops, bacteria, algae, protozoa, fungi, crustaceans, annelids, platyhelminths, nematodes, bivalves, gastropods, and fish, reported in several updated reviews (Tangaa et al. 2016; Rocha et al.

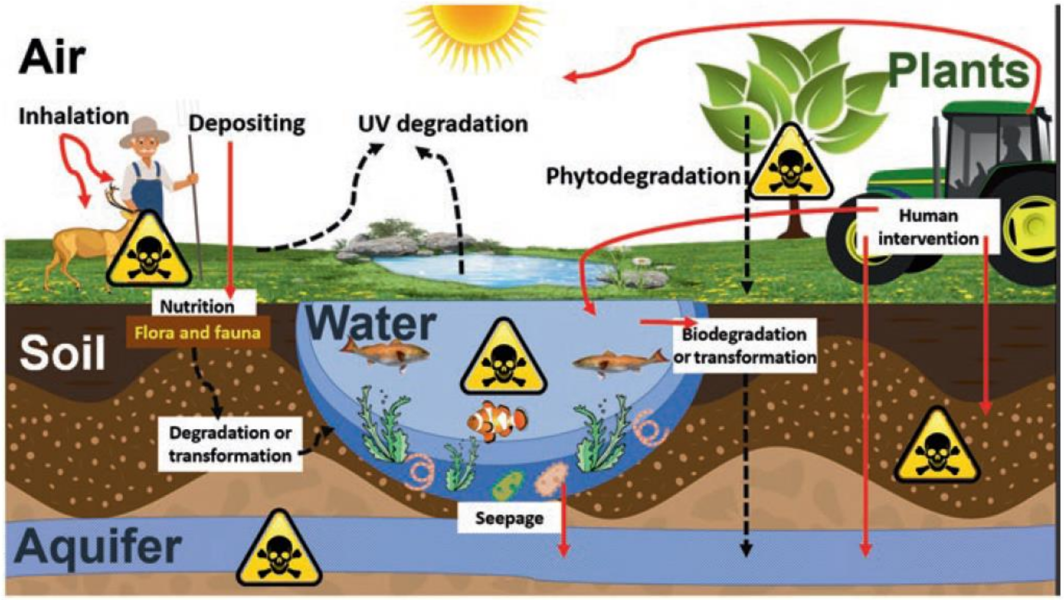

Fig. 9.1 Entry or migration (*solid red lines*) and transformation or degradation (*dashed black lines*) of engineered nanoparticles (ENPs) in different ecosystems. The *danger symbols* denote organisms or systems for which evidence regarding nanoparticle toxicity has been reported (see Tables 9.1, 9.2, and 9.3). *UV* ultraviolet

2017; Karimi et al. 2018; Tan et al. 2018). It is suggested that precise and standard tests should be utilized to assess the long-term effects of acute and chronic exposure to different ENMs existing in food systems before mass production. Overall, the available knowledge indicates an urgent need to study the effects of ENPs on humans and on the environment, in order to develop environmentally sustainable nanotechnologies.

3 Effects of Engineered Nanomaterials on Plants

Reports indicate that ENMs affect plants differently at the physiological, biochemical, nutritional, and genetic levels, while effects on growth, physiological and biochemical traits, production, and food quality, among other things, have been reported. However, our understanding of the dynamics of interactions between plants and ENMs is not clear enough yet (Rajput et al. 2018; De la Rosa et al. 2017; Rizwan et al. 2017; Zuverza-Mena et al. 2017). This review clearly confirms the existence of toxic effects of ENMs on cultivated crop plants through inhibition of seed germination, decreases in root and shoot lengths, reductions in photosynthesis and respiration rates, and morphological as well as enzymatic changes (Table 9.1). However, benefic effects of ENMs on plants have also been reported (Table 9.2).

Table 9.1 Negative effects of different engineered nanoparticles (ENPs) on plant species

Types and sizes (nm) of ENP	Species	Effects	Reference
CeO_2 (8)	*Triticum aestivum*	Root changes; decreased chlorophyll content and starch grain size in endosperm	Du et al. (2015)
CuO (100–200)	*Lactuca sativa*	Effects on seed germination, vigor index, and fresh weight; root length reduced by 49%	Hong et al. (2015)
CuO (0–80)	*Coriandrum sativum*	Effects on germination rate and shoot elongation	Zuverza-Mena et al. (2015)
CuO (<1200 to >2100)	*Daucus carota*	Reduced shoot biomass and restricted Cu accumulation in taproot periderm	Ebbs et al. (2016)
NiO (<100)	*Hordeum vulgare*	Decreased leaf surface, chlorophyll, and carotenoids	Soares et al. (2016)
ZnO (15)	*Triticum aestivum*	Reduced photosynthetic efficiency, inhibited antioxidant activity	Tripathi et al. (2017)
Ag (12 ± 9)	*Capsicum annuum*	Decreased plant growth	Vinkovic et al. (2017)
$AgNO_3$ (61.2 ± 33.9)	*Nicotiana tabacum*	Oxidative stress and changes in chloroplast size	Cvjetko et al. (2018)
CuO (<50) and ZnO (<100)	*Raphanus sativus*	Reduced root length, shoot length, and biomass	Singh and Kumar (2018)

Table 9.2 Positive effects of different engineered nanoparticles (ENPs) on plant species

Types and sizes (nm) of ENP	Species	Effects	Reference
TiO_2 (25)	*Solanum lycopersicum*	Promoted plant height, root length, and biomass	Raliya et al. (2015)
Ag (200–800)	*Trigonella foenum-graecum*	Enhanced plant growth and diosgenin synthesis	Jasim et al. (2016)
Fe_3O_4 (17 ± 3.9)	*Zea mays*	Increased germination index	Li et al. (2016)
Fe_2O_3 (10)	*Solanum lycopersicum*	Increased root and shoot lengths with 50–200 mg L^{-1} solution	Shankramma et al. (2017)
Cu-grown carbon nanofibers (95)	*Cicer arietinum*	Increased germination rate, shoot and root lengths, and chlorophyll and protein content	Ashfaq et al. (2017)
Nano-γPGA/CS-GA_3 (134 ± 9)	*Phaseolus vulgaris*	Increased leaf area and induced root development (including lateral root formation)	Pereira et al. (2017)
Ag^+ bentonite (1.5)	*Avena byzantina*	Increase root growth	Tomacheski et al. (2017)
ZnO (NR)	*Gossypium hirsutum*	Increased plant growth, biomass, chlorophyll, carotenoids, protein content, superoxide dismutase, and peroxidase	Venkatachalam et al. (2017)
ZnO (NR)	*Carthamus tinctorius*	Increased guaiacol peroxidase, polypeptide oxidase, dehydrogenase, and malondialdehyde	Hafizi and Nasr (2018)
TiO_2 (28.78)	*Vicia faba*	Increased shoot length, leaf area, and root dry weight	Latef et al. (2018)

γPGA poly(γ-glutamic acid), *CS* chitosan, *NR* not reported.

3.1 *Effects of Engineered Nanomaterials on Common Bean (*Phaseolus vulgaris *L.)*

3.1.1 Experimental Site

This study was carried out in a greenhouse at the Programa de Sustentabilidad de los Recursos Naturales y Energía del Cinvestav-Saltillo, located in Saltillo, Coahuila, Mexico. According to the Köppen climate classification, this area has a semiarid hot climate (BSh). According to the United Nations Food and Agriculture Organization and the United Nations Educational, Scientific, and Cultural Organization (FAO/UNESCO) soil classification system, the soil is a haplic xerosol.

3.1.2 Biological Materials

Common bean seeds were donated by INIFAP-Celaya, Mexico. All seeds were kept in the dark at 4 °C until use.

3.1.3 Nanomaterials

Nanoparticles of magnetite, ferrihydrite, and hematite were manufactured, while nanoparticles of zinc oxide and titanium dioxide were purchased from Materiales Nanoestructurados SA de CV (San Luis Potosí, Mexico). The crystallographic system is cubic for magnetite, tetragonal for zinc oxide and hexagonal for ferrihydrite, hematite, and titanium dioxide. X-ray diffraction was conducted to verify the pure phase samples, and the magnetic properties of the samples were measured using a MicroMag™ 2900 Alternating Gradient Magnetometer.

3.1.4 Cultivation of Plants in the Greenhouse

The full experimental setup was repeated three times. The first experiment was carried out from January to May 2013, the second one from February to June 2013, and the third one from March to July 2013. Sixty subsamples of 3500 g of soil [i.e., five kinds of nanoparticle (nano-Fe_3O_4, nano-$FeOOH \cdot xH_2O$, nano-α-Fe_2O_3, nano-ZnO, and nano-TiO_2) in triplicate × four concentrations] were added to square plastic pots whose length × width × height were 17 × 15 × 17 cm. Five treatments (nanoparticles) at four concentrations (0, 1, 3, and 6 g L^{-1}) were applied to the soil during irrigation, so we sprayed each plastic pot with 500 mL of a 0, 1, 3, or 6-g L^{-1} nanoparticle suspension throughout the experiment. Three seeds of common bean were planted in 180 plastic pots [i.e., five nanoparticles in triplicate × four concentrations in three experiments]. The seeds were placed at a 2-cm depth in each plastic pot. Five days after planting, the seedlings were thinned to one plant per plastic pot. The plastic pots were placed in the greenhouse for 120 days. A plastic container was placed under each plastic pot to collect drained liquid. However, the irrigation was well controlled, so no leaching was observed. Thirty, 60, and 120 days after sowing, three plastic pots were selected at random from each treatment and each concentration. The entire soil column was removed from the plastic pot, and samples were taken from the 0- to 7.5-cm depth and from the 7.5- to 15-cm depth, with care so as not to damage the root structure. The roots were separated from the shoots, and the root and shoot length were measured. The roots and shoots were dried at 70 °C, weighed, and analyzed for Ti, Fe, Zn, and total N. The soils from the 0- to 7.5-cm and 7.5- to 15-cm depths were analyzed for pH, electrical conductivity (EC), Ti, Fe, and Zn. The amount of chlorophyll was quantified every 2 days after sowing, beginning on day 15. The temperature and moisture content inside the greenhouse during the experiment were 24 °C and 35–45%, respectively.

3.1.5 Chemical Analyses

The pH was measured in 1:2.5 soil or wastewater sludge/H_2O suspension, using a 716 DMS Titrino pH meter (Metrohm Ltd., Herisau, Switzerland) fitted with a glass electrode. The EC was determined in a 1:5 soil/H_2O suspension. The organic C in the soil was measured using a TOC-VCSH total organic carbon analyzer (Shimadzu, Columbia, MD, USA). The inorganic C was determined by adding 5 mL of 1-M hydrogen chloride (HCl) solution to 1 g of air-dried soil and trapping the evolved CO_2 in 20 mL of 1-M NaOH. The total N in the soil, root, and shoot was measured by the Kjeldahl method using concentrated H_2SO_4, K_2SO_4, and $CuSO_4$ to digest the sample. The soil particle size distribution was defined by the hydrometer method. The water-holding capacity (WHC) was measured in 6.5 kg of soil placed in a polyvinyl chloride (PVC) tube (length 50 cm, diameter 16 cm), water saturated, stoppered with a PVC ring, and left to stand overnight to drain freely (WHC = [(water-saturated soil − soil dried at 105 °C)/soil dried at 105 °C] × 1000). The amount of chlorophyll was measured with a Minolta SPAD-502 chlorophyll meter. Fe, Ti, and Zn were determined by inductively coupled plasma mass spectrometry (ICP–MS).

3.1.6 Statistical Analyses

The data were subjected to an analysis of variance (ANOVA) and means were compared with the Tukey test, using Statistical Analysis System (SAS) software version 8.0 for Windows. Soil and plant characteristics were subjected to one-way ANOVA using a general linear model procedure (PROC GLM) to test for significant differences ($p < 0.05$) between treatments. The methodology used for the principal component analysis (PCA) has previously been described by Fernandez-Luqueno et al. (2016) and Medina-Pérez et al. (2018). All analyses were performed using the SAS statistical package. All data presented are the means of three replicates in soil from three different plots, and the whole experiment was repeated three times (n = 27) with sampling after 30, 60, and 120 days.

3.1.7 Results and Discussion

None of the five kinds of nanoparticle used in this experiment (magnetite, ferrihydrite, hematite, zinc oxide, and titanium dioxide) significantly modified the chlorophyll content of common bean plants, as evidenced by the Soil Plant Analysis Development (SPAD) unit values (see Fig. 9.2). However, the nanoparticles of magnetite, ferrihydrite, hematite, zinc oxide, and titanium dioxide significantly modified at least one plant characteristic or one yield component of common bean. The nanoparticles containing Fe (magnetite, ferrihydrite, and hematite) were those that significantly affected more crop characteristics such as the total N in the roots or shoots, number of pods, dry weight of pods, number of seeds, and yield of common bean. These findings are an important factor to take into account with regard to the applicability of nanoparticles for long-term use in crops, but selection of the most

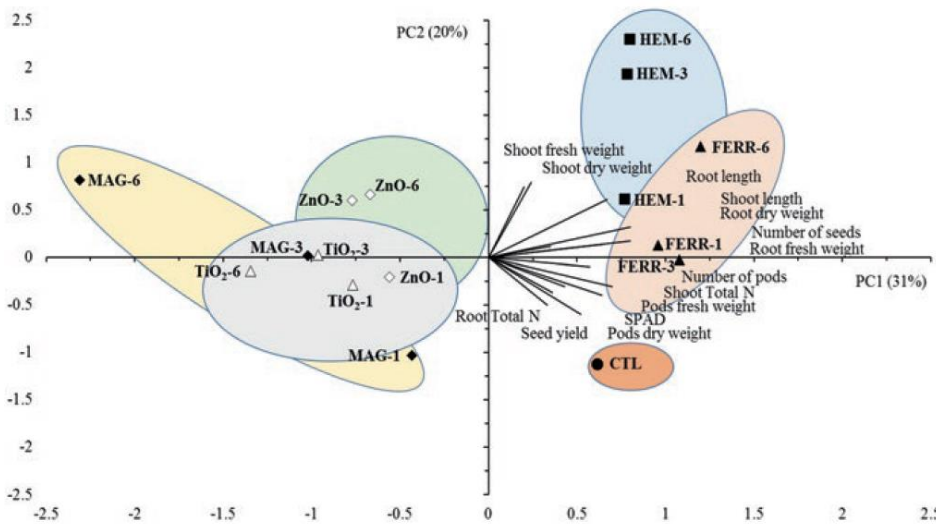

Fig. 9.2 Principal component (PC) analysis of characteristics of bean plants (*Phaseolus vulgaris* L.) cultivated in agricultural soil irrigated with 500 mL of a 0, 1, 3, or 6-g L^{-1} nanoparticle suspension. Nanoparticles of Fe_3O_4, $FeOOH{\cdot}xH_2O$, α-Fe_2O_3, ZnO, and TiO_2 were used. The data are the mean values from three square plastic pots with 3.5 kg of dry soil in each one, with three different soils and three experiments (i.e., $n = 27$). Each whole experiment lasted for 120 days. The first two factors explained 51% of the variation. *CTL* control, *FERR-1* 500 mL of a 1-g $FeOOH{\cdot}xH_2O$ nanoparticle suspension, *FERR-3* 500 mL of a 3-g $FeOOH{\cdot}xH_2O$ nanoparticle suspension, *FERR-6* 500 mL of a 6-g $FeOOH{\cdot}xH_2O$ nanoparticle suspension, *HEM-1* 500 mL of a 1-g α-Fe_2O_3 nanoparticle suspension, *HEM-3* 500 mL of a 3-g α-Fe_2O_3 nanoparticle suspension, *HEM-6* 500 mL of a 6-g α-Fe_2O_3 nanoparticle suspension, *MAG-1* 500 mL of a 1-g Fe_3O_4 nanoparticle suspension, *MAG-3* 500 mL of a 3-g Fe_3O_4 nanoparticle suspension, *MAG-6* 500 mL of a 6-g Fe_3O_4 nanoparticle suspension, *SPAD* Soil Plant Analysis Development, *TiO_2-1* 500 mL of a 1-g TiO_2 nanoparticle suspension, *TiO_2-3* 500 mL of a 3-g TiO_2 nanoparticle suspension, *TiO_2-6* 500 mL of a 6-g TiO_2 nanoparticle suspension, *ZnO-1* 500 mL of a 1-g ZnO nanoparticle suspension, *ZnO-3* 500 mL of a 3-g ZnO nanoparticle suspension, *ZnO-6* 500 mL of a 6-g ZnO nanoparticle suspension

appropriate nanoparticles at the most appropriate concentration is important for realization of greater benefits and agrosustainability. Additionally, there is a need to generate more data on chronic effects of long-term and concentrated exposure of plants to nanoparticles, as this is important for better understanding of the potential hazards or risks of these nanoparticles. More studies are also needed to identify the greatest potential of nanoparticles in the rural sector and in the agro-food industry worldwide.

3.2 *Effects of Engineered Nanomaterials on Maize (Zea mays L.)*

The experimental site, biological materials, nanomaterials, procedures for cultivation of plants in the greenhouse, chemical analyses, and statistical analyses were similar to those described in Sect. 3.1.

3.2.1 Results and Discussion

Magnetite, ferrihydrite, and hematite significantly modified the chlorophyll content of maize plants, as evidenced by the SPAD unit values, while zinc oxide and titanium dioxide did not significantly modify any plant characteristic or yield component at the physiological maturity of the crop (see Fig. 9.3). The nanoparticles containing Fe (magnetite, ferrihydrite, and hematite) were those that significantly increased crop characteristics such as the total N in the roots or shoots, but not the yield of maize.

4 Effects of Engineered Nanomaterials on Earthworms

Earthworms live in almost all kinds of soil worldwide and may represent 60–80% of the total soil biomass. Earthworms play a key role in soil ecosystems because they contribute to pedogenesis, water regulation, nutrient cycling, aeration, removal of contaminants, and soil structure formation. Although earthworms accelerate the removal of organic and inorganic pollutants from soils, their activity may be inhibited when excessive amounts of pollutants are discarded in their habitat.

Despite the large amount of research into the potential applications of nanotechnology conducted in recent years, relatively little has been done to assess the potential risks of nanoparticles for earthworms. Stewart et al. (2013) stated that chemical modification of cadmium selenide QDs protected *Eisenia andrei* and reduced the bioaccumulation of nanoparticles by earthworms. Other experiments on the nanotoxicity of nanoparticles to *E. andrei* were carried out by Romero-Freire et al. (2017). They reported that survival, weight change, and reproduction were affected

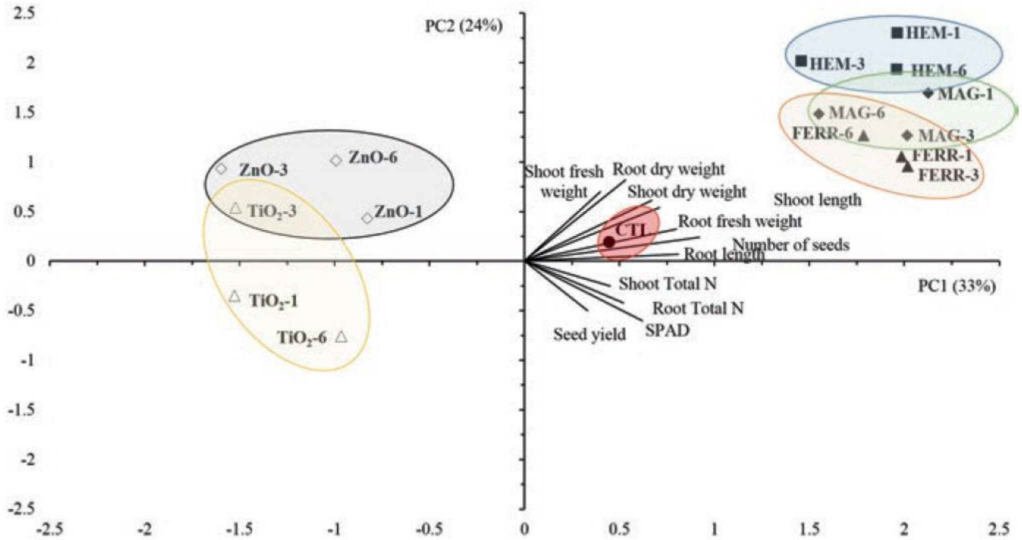

Fig. 9.3 Principal component (PC) analysis of characteristics of maize (*Zea mays* L.) cultivated in agricultural soil irrigated with 500 mL of a 0, 1, 3, or 6-g L^{-1} nanoparticle suspension. Nanoparticles of Fe_3O_4, $FeOOH{\cdot}xH_2O$, α-Fe_2O_3, ZnO, and TiO_2 were used. The data are the mean values from three square plastic pots with 3.5 kg of dry soil in each one, with three different soils and three experiments (i.e., $n = 27$). Each whole experiment lasted for 120 days. *CTL* control, *FERR-1* 500 mL of a 1-g $FeOOH{\cdot}xH_2O$ nanoparticle suspension, *FERR-3* 500 mL of a 3-g $FeOOH{\cdot}xH_2O$ nanoparticle suspension, *FERR-6* 500 mL of a 6-g $FeOOH{\cdot}xH_2O$ nanoparticle suspension, *HEM-1* 500 mL of a 1-g α-Fe_2O_3 nanoparticle suspension, *HEM-3* 500 mL of a 3-g α-Fe_2O_3 nanoparticle suspension, *HEM-6* 500 mL of a 6-g α-Fe_2O_3 nanoparticle suspension, *MAG-1* 500 mL of a 1-g Fe_3O_4 nanoparticle suspension, *MAG-3* 500 mL of a 3-g Fe_3O_4 nanoparticle suspension, *MAG-6* 500 mL of a 6-g Fe_3O_4 nanoparticle suspension, *SPAD* Soil Plant Analysis Development, *TiO_2-1* 500 mL of a 1-g TiO_2 nanoparticle suspension, *TiO_2-3* 500 mL of a 3-g TiO_2 nanoparticle suspension, *TiO_2-6* 500 mL of a 6-g TiO_2 nanoparticle suspension, *ZnO-1* 500 mL of a 1-g ZnO nanoparticle suspension, *ZnO-3* 500 mL of a 3-g ZnO nanoparticle suspension, *ZnO-6* 500 mL of a 6-g ZnO nanoparticle suspension

by both Zn-NP and $ZnCl_2$, but they could not explain the differences in earthworm toxicity. Similar studies were done by Swiatek et al. (2017) to evaluate the effects of Zn-NP or $ZnCl_2$ on reproduction of *E. andrei*, but zinc was efficiently regulated by the earthworms in all treatments.

Enchytraeus crypticus has been also studied to determinate the toxicity of ZnO-NP to annelids (Hrda et al. 2016). It was found that toxicity was clearly dependent on the size of the ZnO-NP agglomerates and the technique used for exposure medium preparation, but it was not correlated with the ZnO-NP concentration. The survival and composition of the gut microflora of *Eisenia fetida* grown in soil polluted with Zn-NP have been also analyzed (Yausheva et al. 2016). It was reported that Zn-NP decreased the diversity of bacteria belonging to the taxon Firmicutes and increased the proportion of Proteobacteria. Other authors have found evidence regarding ENM toxicity to earthworms in soils (Table 9.3).

Table 9.3 Effects of different engineered nanoparticles (ENPs) on earthworm species

Types and sizes (nm) of ENP	Species	Effects	Reference
$TiSiO_4$ (<50)	*Eisenia andrei* and *Folsomia candida*	No effect on either species	Bouguerra et al. (2017)
MoO_3 (92 ± 0.3)	*Eisenia fetida*	Mortality and decreased weight	Lebedev et al. (2016)
C-nZVI (NR)	*Eisenia fetida*	No effect	Yirsaw et al. (2016)
$AgNO_3$ (NR)	*Allolobophora chlorotica*	Mortality	Brami et al. (2017)
Ag-NP (30 ± 2) and $AgNO_3$ (34 ± 3)	*Eisenia andrei*	Reduced number of juveniles; cocoons not viable (not hatched)	Jesmer et al. (2017)
ZnO and $ZnCl_2$ (20–40 nm)	*Eisenia andrei*	Effects on survival, weight, and reproduction	Romero-Freire et al. (2017)

Ag-NP silver nanoparticles, *C-nZVI* coated nanoscale zero-valent iron, *NR* not reported

4.1 Effects of Nanoparticles of Hematite, Zinc Oxide, and Titanium Dioxide on Eisenia fetida

The experimental site and nanomaterials used in this experiment were similar to those described in Sects. 3.1 and 3.2.

4.1.1 Soil Preparation

The soil was taken to the laboratory and passed separately through a 5-mm sieve, adjusted to 40% WHC by addition of distilled water (H_2O), and conditioned at 22 ± 2 °C for 10 days in drums containing a beaker with 1000 mL of 1-M sodium hydroxide (NaOH) solution to trap the evolved CO_2, and a beaker with 1000 mL of distilled H_2O to avoid desiccation of the soil. After this process, the soil was tyndallized.

4.1.2 Vermicompost Preparation

The vermicompost used to feed the earthworms was obtained from the worm culture maintained at our facility for 2 months, which was kept on precomposted organic material bedding. Thereafter, the material obtained was tyndallized to remove any organisms that could be harmful to the earthworms.

4.1.3 *Eisenia fetida* Culture

All earthworms used in the present study came from a culture of *E. fetida* maintained at our facility. The culture is kept on bedding of precomposted organic kitchen waste.

4.1.4 Experimental Setup

One hundred and sixty-eight subsamples of 200 g of dry soil [i.e., 14 treatments in triplicate × four destructive sample dates (0, 20, 40, and 60 days after the onset of the experiment)] were added to 900-mL amber glass jars (length 18 cm, diameter 10 cm). α-Fe_2O_3-NP, ZnO-NP, or TiO_2-NP were applied to the soil at three increasing concentrations (0.0, 0.15, and 0.3 g kg^{-1} of dry soil), so six chemical suspensions of nanoparticles were prepared (three nanoparticle types × two concentrations) in distilled water, and they were sonicated for 30 min before use; after the sonication the nanoparticle suspensions were added to the earthworm food (vermicompost or Quaker® oats), and after the food was added it was completely mixed with the soil. The experiment was carried out under plant growth chamber conditions; the average temperature was 22 ± 2 °C, and the photoperiod was 12 h light and 12 h dark. In a completely randomized design, each experimental unit was prepared, incubated, and sampled independently. Ten *E. fetida* earthworms were used in each experimental unit of this research. At the onset of the experiment, 35 g of dry vermicompost was added to each amber glass jar to feed the earthworms. Additionally, 30 and 50 days after the onset of the experiment, 35 g of tyndallized Quaker® oats was added to feed the earthworms. Fourteen treatments were applied to the soil. The aerobic incubation experiment lasted for 60 days, in which four destructive and random samplings were performed on days 0, 20, 40, and 60. On each sampling day, adult earthworms, cocoons, and juveniles were hand sorted and counted.

4.1.5 Chemical Characterization of Soil, Vermicompost, and Biochemical Analyses

The methodologies used for chemical analysis of the soil, vermicompost, and earthworms were similar to those described in Sects. 3.1 and 3.2.

4.1.6 Data Analysis and Statistical Methods

The methodologies used for statistical analysis were similar to those described in Sects. 3.1 and 3.2. All data presented are the means of three replicates × four destructive sample dates (0, 20, 40, and 60 days after the onset of the experiment) × two consecutive experiments carried out in a plant growth chamber (i.e., $n = 24$).

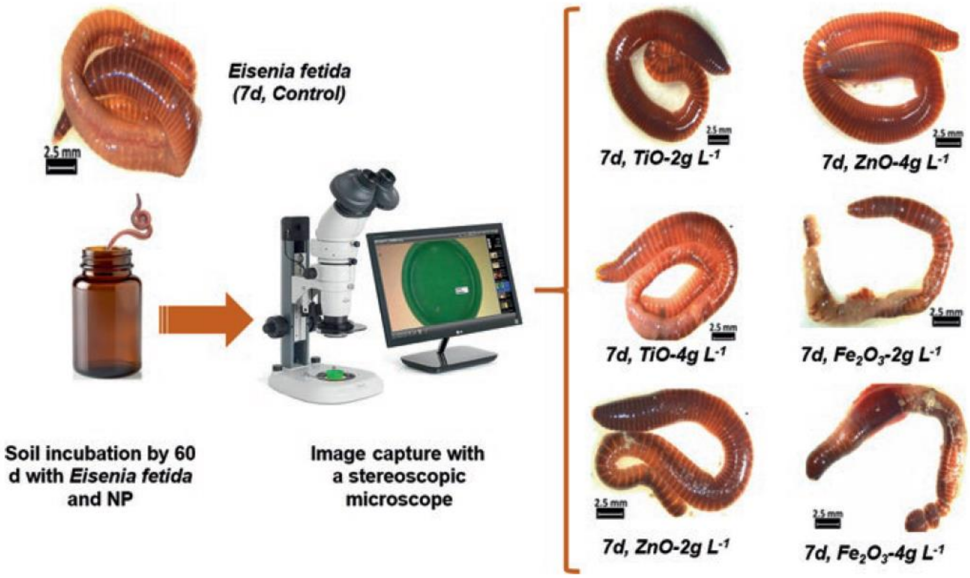

Fig. 9.4 Physical damage in the body of *Eisenia fetida* at 7 days after treatment with nanoparticles (NP)

4.1.7 Results and Discussion

Physical damage was detected in earthworms exposed to increasing doses of Fe_2O_3-NP. The main detected types of damage were inflammation and explosion in certain areas of the earthworm's body at 14 days after the onset of the experiment (Fig. 9.4). Seven days after the onset of the experiment, earthworms treated with Fe_2O_3-NP died.

Hu et al. (2010) evaluated the toxicity of nanoparticles of TiO_2 and ZnO to the earthworm *E. fetida* in soil. Artificial soil systems containing distilled water and 0.1, 0.5, 1.0, or 5.0 g kg^{-1} of nanoparticles were prepared, and earthworms were exposed for 7 days. It was found that Ti and Zn were bioaccumulated and that mitochondria were damaged at the highest dose in soil (5.0 g kg^{-1}). The activity of cellulase was significantly inhibited when organisms were exposed to 5.0 g kg^{-1} of ZnO nanoparticles. This study demonstrated that both TiO_2-NP and ZnO-NP exert harmful effects on *E. fetida* when their levels are higher than 1.0 g kg^{-1} in soil, and the toxicity of ZnO-NP was greater than that of TiO_2-NP.

5 Effects of Engineered Nanomaterials on Microorganisms

The broad variety of applications of ENMs has led to their unusual and widespread distribution in several environmental sectors, with different effects on living organisms. ENMs applied for in situ remediation of water or soil inevitably interact with various microbes at the remediation sites directly or indirectly (Xie et al. 2017;

Lefevre et al. 2016). Studies that refer to microbial communities are central not only in soil sciences but also in all related disciplines. This last statement requires knowledge of which microorganisms are responsible for specific processes. According to Blagodatskaya and Kuzyakov (2013), microbial communities in soils consist of an extensive range of organisms in four physiological states: (1) active; (2) potentially active; (3) dormant; and (4) dead.

To date, over 80,000 species of fungi have been described that live in soil, but many more remain undiscovered, considering that the total fungal diversity is estimated at 1.5 million species (Hawksworth 1991). It is well known that 1 g of soil may contain approximately one million individual fungi, while the fungal biomass may amount to 2.5–5 t ha^{-1}.

In agricultural soils, most of the biological activity occurs in the top 20 cm (the plow layer), while in noncultivated soils, most of the biological activity occurs in the top 5 cm of soil. Diversity of soil organisms is essential for the maintenance of productive soils because soil organisms are responsible for a range of ecological functions and ecosystem services. Therefore, excessive reduction of species with critical features might result in severe effects, including long-term degradation of soils, changes in the landscape, decreasing soil resilience, and loss of agricultural productivity. It has to be remembered that soil health, soil quality, and soil resilience are all fundamental to sustain the productivity and viability of agricultural systems throughout the world.

Microbial communities play a significant and relevant role regarding greenhouse gas (GHG) emissions worldwide. GHG emissions result from complex interactions between abiotic drivers and multiple microbial metabolic processes. Mechanisms controlling CO_2, CH_4, and N_2O production have been well characterized in both oxisol and permafrost (i.e., in all types of soil worldwide).

In the last decade, several publications have reported fragments of information about the interaction, detection, uptake, and translocation of ENMs in microorganisms, and several papers have described negative effects of ENMs on microbial communities (Table 9.4).

5.1 *Microbial Communities in a Soil Amended with Engineered Nanomaterials*

The experimental site, nanomaterials, chemical analyses, and statistical analyses were similar to those described in Sect. 3.1.

5.2 *Experimental Setup and Treatments*

The soil was taken to the greenhouse, sieved (<5 mm), air dried, and characterized. One week before the onset of the experiment, the soil was divided into two equal parts and adjusted to field capacity by addition of distilled water (H_2O). Half of the

Table 9.4 Negative effect of different engineered nanoparticles (ENPs) on soil microorganisms

Types and sizes (nm) of ENP	Species	Effects	Reference
Ag (1–10)	*Acidobacteriaceae bacterium* Ellin5095 (AY234512.1), *Acidobacteriaceae bacterium* Ellin311 (AF498693.1), *Acidobacteriaceae bacterium* Ellin310 (AF498692.1), and other species	Decreased microbial community	Carbone et al. (2014)
ZnO (17)	*Acinetobacter baumanni*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and other microorganisms	Ultrastructural changes	Aal et al. (2015)
TiO_2 (15) and CeO_2 (10)	*Azotobacter*	Reduced abundance of functional bacteria and enzymatic activity	Chai et al. (2015)
Ag (20)	Different microorganisms in *Zea mays* rhizosphere	Effects on community composition	Sillen et al. (2015)
Fe_3O_4 (NR)	AMF in *Zea mays* rhizosphere	Decreased soil bacterial abundance and community composition shifted	Cao et al. (2016)
Ag (50)	*Nitrosomonas europea*	Damaged cell wall of *N. europea*, disintegrated nucleoids, and condensed next to cell membrane	Wang et al. (2017)

AMF arbuscular mycorrhizal fungi, *NR* not reported

soil was adjusted to 40% WHC (considered the conditioned soil samples) and pre-incubated for 7 days in drums containing a beaker with 1000 mL of 1-M NaOH solution to trap the evolved CO_2, and a beaker with 500 mL of distilled H_2O to avoid desiccation of the soil. The drums were opened every day to avoid anaerobic conditions. Thereafter, 20 g of soil was amended with ENMs (nano-Fe_3O_4, nano-ZnO, or nano-TiO_2) at 0, 1, 3, and 6 g kg^{-1} of dry soil. After 0, 30, 60, and 90 days, three soil subsamples were selected at random from each treatment and plot (n = 9) and the number of viable soil microorganisms (i.e., heterotrophic bacteria, fungi, and actinomycetes) was determined as colony-forming units (CFUs).

The numbers of heterotrophic bacteria and actinomycetes were determined by culturing in a mineral salt medium, while fungi were counted using the Martin medium. Culture media were prepared in sterile conditions, autoclaved, and poured into the petri dish bottom. We used the standard plate count technique to determine the number of microorganisms. Bacteria and actinomycetes were counted in 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions, while fungi microorganisms were counted in 10^{-3}, 10^{-4}, and 10^{-5} dilutions.

5.3 Results and Discussion

The CFUs of bacteria and actinomycetes decreased significantly, modified by ENMs (Fig. 9.5a, b). However, ZnO-NP increased the CFU of fungi. Asadishad et al. (2018) stated that TiO_2-NP slightly decreased enzyme activity in agricultural soil. However, they also found that Illumina MiSeq sequencing of microbial communities indicated a shift in soil microbial community composition upon exposure to high doses of metal ions or Ag-NP, and a negligible shift in the presence of TiO_2-NP. In another study, ZnO-NP demonstrated adverse effects on C transformations (but not on N transformations) and adverse effects on dehydrogenase and phosphatase activities in natural soil (Garcia-Gomez et al. 2015).

Liu et al. (2018) studied the impact of wastewater effluent (WE) containing aged nanoparticles. They established a soil microecosystem including a microbiome, four *Arabidopsis thaliana* plants, and three *E. fetida* earthworms, for a duration of 95 days. Although the microbial biomass, carbon, and nitrogen were not significantly reduced, the population distribution of the microbial communities was shifted in WE-irrigated soil compared with the control soil. The abundance of cyanobacteria (cyanophyta) was increased by 12.5% in the WE-irrigated soil, manifested mainly by an increase in *Trichodesmium* spp., and the abundance of unknown archaea was increased from 26.7% in the control soil to 40.5% in the WE-irrigated soil (Liu et al. 2018).

Enough evidence has been found that ENMs significantly modify microbial communities in soils and also change some parameters of other soil organisms such

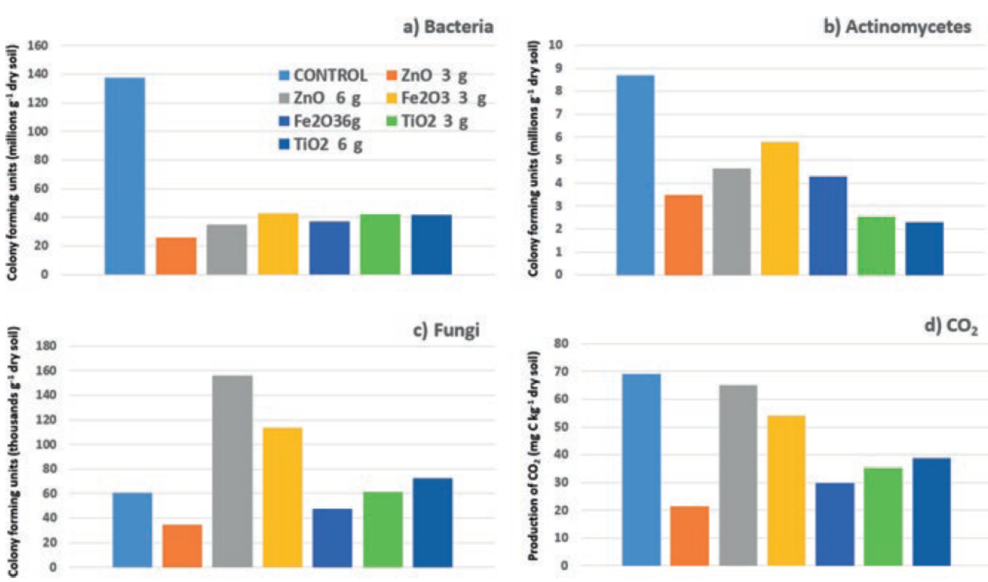

Fig. 9.5 Colony-forming units of soil microorganisms and production of CO_2. (**a**) Bacteria. (**b**) Actinomycetes. (**c**) Fungi. (**d**) Production of CO_2. The data were pooled for four sampling dates in triplicate × three soil sites (i.e., $n = 36$)

as plants or earthworms. Therefore, regulations regarding the use of ENMs and their spread in the environment must be implemented to avoid damage to ecological or human health.

6 Conclusion

Overall, the available knowledge indicates an urgent need to synthesize environmentally sustainable ENMs. In addition, it is suggested that precise and standardized tests should be utilized to assay the long-term effects of acute and chronic exposure to different nanoparticles existing in food systems before mass production and utilization of nanoparticles in the food industry or in emerging technologies, in order to avoid the spread of unregulated or untested ENMs.

Some nanoparticles could have harmless applications. For instance, an evaluation of studies of biologically active nanoparticles provides guidance for the synthesis of nanoparticles with the goal of developing new antibiotics/antifungals to combat microbial resistance. However, the current information leaves no doubt that there are still many aspects in need of additional investigations for us to fully understand the effects of ENMs in organisms. In plants and other organisms of agronomic interest, little is known about the transgenerational effects of ENM exposure and the changes at the agronomical and physiological levels.

Since ENMs have been found in edible tissues, it is expected that they will be present in the food chain; thus, studies on their trophic transfer are required. Overall, nanoscience and nanotechnology require transdisciplinary work by scientists from different areas to study the potential toxicity of ENMs prior to their use or spread in ecosystems.

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References

Aal NA, Al-Hazmi F, Al-Ghamdi AA, Al-Ghamdi AA, El-Tantawy F, Yakuphanoglu F (2015) Novel rapid synthesis of zinc oxide nanotubes via hydrothermal technique and antibacterial properties. Spectrochim Acta A 135:871–877

Asadishad B, Chahal S, Akbari A, Cianciarelli V, Azodi M, Ghoshal S, Tufenkji N (2018) Amendment of agricultural soil with metal nanoparticles: effects on soil enzyme activity and microbial community composition. Environ Sci Technol 52(4):1908–1918

Ashfaq M, Verma N, Khan S (2017) Carbon nanofibers as a micronutrient carrier in plants: efficient translocation and controlled release of Cu nanoparticles. Environ Sci Nano 4:138

Blagodatskaya E, Kuzyakov Y (2013) Active microorganisms in soil: critical review of estimation criteria and approaches. Soil Biol Biochem 67:192–211

Bouguerra S, Gavina A, Ksibi M, Rasteiro MG, Rocha-Santos T, Pereira R (2017) Ecotoxicity of titanium silicon oxide ($TiSiO_4$) nanomaterial for terrestrial plants and soil invertebrate species. Ecotoxicol Environ Saf 129:291–301

Brami C, Glover AR, Butt KR, Lowe CN (2017) Effects of silver nanoparticles on survival, biomass change and avoidance behaviour of the endogeic earthworm *Allolobophora chlorotica*. Ecotoxicol Environ Saf 141:64–69

Cao J, Fen Y, Lin X, Wang J (2016) Arbuscular mycorrhizal fungi alleviate the negative effects of iron oxide nanoparticles on bacterial community in rhizospheric soils. Front Environ Sci 4:10. https://doi.org/10.3389/fenvs.2016.00010

Carbone S, Vittori Antisari L, Gaggiaa F, Baffonia L, Di Gioiaa D, Vianelloa G, Nannipieri P (2014) Bioavailability and biological effect of engineered silver nanoparticles in a forest soil. J Hazard Mater 280:89–96

Chai H, Yao J, Sun J, Zhang C, Liu W, Zhu M, Ceccanti B (2015) The effect of metal oxide nanoparticles on functional bacteria and metabolic profiles in agricultural soil. Bull Environ Contam Toxicol 94:490–495

Cornelis G, Doolette C, Thomas M, McLaughlin MJ, Kirby JK, Beak DG, Chittleborough D (2012) Retention and dissolution of engineered silver nanoparticles in natural soils. Soil Sci Soc Am J 76(3):891–902

Cvjetko P, Zovko M, Štefanić PP, Biba R, Tkalec M, Domijan AM, Vrček IV, Letofsky-Papst IP, Šikić S, Balen B (2018) Phytotoxic effects of silver nanoparticles in tobacco plants. Environ Sci Pollut Res 25:5590

De la Rosa G, Garcia-Castaneda C, Vazquez-Nunez E, Alonso-Castro AJ, Basurto-Islas G, Mendoza A, Cruz-Jimenez G, Molina C (2017) Physiological and biochemical response of plants to engineered NMs: implications on future design. Plant Physiol Biochem 110:226–235

Du W, Gardea-Torresdey JL, Ji R, Yin Y, Zhu J, Peralta-Videa JR, Guo H (2015) Physiological and biochemical changes imposed by CeO_2 nanoparticles on wheat: a life cycle field study. Environ Sci Technol 49:11884–11893

Ebbs SD, Bradfield SJ, Kumar P, White JC, Musante C, Ma X (2016) Accumulation of zinc, copper, or cerium in carrot (*Daucus carota*) exposed to metal oxide nanoparticles and metal ions. Environ Sci Nano 3:114–126

Fernandez-Luqueno F, Lopez-Valdez F, Dendooven L, Luna-Suarez S, Ceballos-Ramirez JM (2016) Why wastewater sludge stimulates and accelerates removal of PAHs in polluted soils? Appl Soil Ecol 101: 1-4

Garcia-Gomez C, Babin M, Obrador A, Alvarez J, Fernandez M (2015) Integrating ecotoxicity and chemical approaches to compare the effects of ZnO nanoparticles, ZnO bulk, and $ZnCl_2$ on plants and microorganisms in a natural soil. Environ Sci Pollut Res 22(21):16803–16813

Gokhale S (2016) Effects of engineered nanomaterials released into the atmosphere. J Hazard Toxic Radioact Waste 20(1):UNSP B4015005

Hafizi Z, Nasr N (2018) The effect of zinc oxide nanoparticles on safflower plant growth and physiology. Eng Technol Appl Sci Res 8(1):2508–2513

Hawksworth DL (1991) The fungal dimension of biodiversity—magnitude, significance, and conservation. Mycol Res 95(6):641–655

Hong J, Rico CM, Zhao L, Adeleye AS, Keller AA, Peralta-Videa JR, Gardea Torresdey JL (2015) Toxic effects of copper-based nanoparticles or compounds to lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*). Environ Sci Process Impacts *17*:177–185. https://doi.org/10.1039/c4em00551a

Hrda K, Oprsal J, Knotek P, Pouzar M, Vlcek M (2016) Toxicity of zinc oxide nanoparticles to the annelid *Enchytraeus crypticus* in agar-based exposure media. Chem Pap 70(11):1512–1520

Hu CW, Li M, Cui YB, Li DS, Chen J, Yang LY (2010) Toxicological effects of TiO_2 and ZnO nanoparticles in soil on earthworm *Eisenia fetida*. Soil Biol Biochem 42(4):586–591

Jasim B, Thomas R, Mathew J, Radhakrishnan EK (2016) Plant growth and diosgenin enhancement effect of silver nanoparticles in Fenugreek (*Trigonella foenum-graecum* L.). Saudi Pharm J 25:443–447

Jesmer AH, Velicogna JR, Schwertfeger DM, Scroggins RP, Princz JI (2017) The toxicity of silver to soil organisms exposed to silver nanoparticles and silver nitrate in biosolids-amended field soil. Environ Toxicol Chem 36:2756–2765. https://doi.org/10.1002/etc.3834

Karimi M, Sadeghi R, Kokini J (2018) Human exposure to nanoparticles through trophic transfer and the biosafety concerns that nanoparticle-contaminated foods pose to consumers. Trends Food Sci Technol 75:139–145

Keller AA, Lazareva A (2014) Predicted releases of engineered nanomaterials: from global to regional to local. Environ Sci Technol Lett 1(1):65–70

Latef AAHA, Srivastava AK, El-sadek MSA, Kordrostami M, Tran L-SP (2018) Titanium dioxide nanoparticles improve growth and enhance tolerance of broad bean plants under saline soil conditions. Land Degrad Dev 29:1065–1073

Lebedev S, Yausheva E, Galaktionova L, Sizova E (2016) Impact of molybdenum nanoparticles on survival, activity of enzymes, and chemical elements in Eisenia fetida using test on artificial substrata. Environ Sci Pollut Res 23(18): 18099-18110.

Lefevre E, Bossa N, Wiesner MR, Gunsch CK (2016) A review of the environmental implications of in situ remediation by nanoscale zero valent iron (nZVI): behaviour, transport and impacts on microbial communities. Sci Total Environ 565:889–901

Li J, Hu J, Ma C, Wang Y, Wu C, Huang J, Xing B (2016) Uptake, translocation and physiological effects of magnetic iron oxide (γFe_2O_3) nanoparticles in corn (*Zea mays* L.). Chemosphere 159:326–334

Liu J, Williams PC, Geisler-Lee J, Goodson BM, Fakharifar M, Peiravi M, Chen D, Lightfoot DA, Gemeinhardt ME (2018) Impact of wastewater effluent containing aged nanoparticles and other components on biological activities of the soil microbiome, *Arabidopsis* plants, and earthworms. Environ Res 164:197–203

Medina-Pérez G, Fernández-Luqueño F, Trejo-Téllez LI, López-Valdez F, Pampillón-González L (2018) Growth and development of common bean (*Phaseolus vulgaris* L.) var. Pinto Saltillo exposed to iron, titanium, and zinc oxide nanoparticles in an agricultural soil. Appl Ecol Environ Res 16(2):1883–1897

Medina-Pérez G, Fernández-Luqueño F, Vazquez-Nuñez E, López-Valdez F, Prieto-Mendez J, Madariaga-Navarrete A, Miranda-Arámbula M (in press) Remediation of polluted soils using nanotechnologies: environmental benefits and risks. Pol J Environ Stud

Parisi C, Vigani M, Rodriguez-Cerezo E (2015) Agricultural nanotechnologies: what are the current possibilities? Nano Today 10(2):124–127

Pereira AES, Sandoval-Herrera IE, Zavala-Betancourt SA, Oliveira HC, Ledezma-Pérez AS, Romero J, Fraceto LF (2017) γ-Polyglutamic acid/chitosan nanoparticles for the plant growth regulator gibberellic acid: characterization and evaluation of biological activity. Carbohydr Polym 157:1862–1873. https://doi.org/10.1016/j.carbpol.2016.11.073

Rajput VD, Minkina T, Suskova S, Mandzhieva S, Tsitsuashvili V, Chapligin V, Fedorenko A (2018) Effects of copper nanoparticles (CuO NPs) on crop plants: a mini review. Bionanoscience 8(1):36–42

Raliya R, Nair R, Chavalmane S, Wang WN, Biswas P (2015) Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (*Solanum lycopersicum* L.) plant. Metallomics 7:1584–1594

Rizwan M, Ali S, Qayyum MF, Ok YS, Adrees M, Ibrahim M, Zia-ur-Rehmand M, Farid M, Abbas F (2017) Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: a critical review. J Hazard Mater 322:2–16

Rocha TL, Mestre NC, Saboia-Morais SMT, Babianno MJ (2017) Environmental behaviour and ecotoxicity of quantum dots at various trophic levels: a review. Environ Int 98:1–17

Romero-Freire A, Lofts S, Martín Peinado FJ, van Gestel CA (2017) Effects of aging and soil properties on zinc oxide nanoparticle availability and its ecotoxicological effects to the earthworm *Eisenia andrei*. Environ Toxicol Chem 36:137–146

Shankramma KS, Yallappa MB, Manjanna SJ (2017) Fe_2O_3 magnetic nanoparticles to enhance *S. lycopersicum* (tomato) plant growth and their biomineralization. Appl Nanosci 6:983–990. https://doi.org/10.1007/s13204-015-0510-y

Sillen WMA, Thijs S, Abbamondi GR, Janssen J, Weyens N, White JC, Vangronsveld J (2015) Effects of silver nanoparticles on soil microorganisms and maize biomass are linked in the rhizosphere. Soil Biol Biochem 91:14–22

Singh D, Kumar A (2018) Investigating long-term effect of nanoparticles on growth of *Raphanus sativus* plants: a trans-generational study. Ecotoxicology 27:23

Soares C, Branco-Neves S, de-Sousa A, Pereira R, Fidalgo F (2016) Ecotoxicological relevance of nano-NiO and acetaminophen to *Hordeum vulgare* L.: combining standardized procedures and physiological endpoints. Chemosphere 165:442–452

Song RS, Qin YW, Suh S, Keller AA (2017) Dynamic model for the stocks and release flows of engineered nanomaterials. Environ Sci Technol 51(21):12424–12433

Stewart DTR, Noguera-Oviedo K, Lee V, Banerjee S, Watson DF, Aga DS (2013) Quantum dots exhibit less bioaccumulation than free cadmium and selenium in the earthworm *Eisenia andrei*. Environ Toxicol Chem 32(6):1288–1294

Swiatek ZM, van Gestel CAM, Bednarska AJ (2017) Toxicokinetics of zinc-oxide nanoparticles and zinc ions in the earthworm *Eisenia andrei*. Ecotoxicol Environ Saf 143:151–158

Tan WJ, Peralta-Videa JR, Gardea-Torresdey JL (2018) Interaction of titanium dioxide nanoparticles with soil components and plants: current knowledge and future research needs—a critical review. Environ Sci Nano 5(2):257–278

Tangaa SR, Selck H, Winther-Nielsen M, Khan FR (2016) Trophic transfer of metal-based nanoparticles in aquatic environments: a review and recommendations for future research focus. Environ Sci Nano 3(5):966–981

Terekhova V, Gladkova M, Milanovskiy E, Kydralieva K (2017) Engineered nanomaterials' effects on soil properties: problems and advances in investigation. In: Ghorbanpour M, Khanuja M, Varma A (eds) Nanoscience and plant–soil systems. Springer, Cham, pp 115–136

Tomacheski D, Pittol M, Simões DN, Ribeiro VF, Santana RMC (2017) Impact of silver ions and silver nanoparticles on the plant growth and soil microorganisms. Global J Environ Sci Manage 4(4):341–350

Tripathi DK, Singh S, Singh S, Srivastava PK, Singh VP, Singh S, Prasad SM, Singh PK, Dubey NK, Pandey AC, Chauhan DK (2017) Nitric oxide alleviates silver nanoparticles (AgNPs)–induced phytotoxicity in *Pisum sativum* seedlings. Plant Physiol Biochem 110:167–177

Venkatachalam P, Priyanka N, Manikandan K, Ganeshbabu I, Indiraarulselvi P, Geetha N, Muralikrishna K, Bhattacharya RC, Tiwari M, Sharma N, Sahi SV (2017) Enhanced plant growth promoting role of phycomolecules coated zinc oxide nanoparticles with P supplementation in cotton (*Gossypium hirsutum* L.). Plant Physiol Chem 110:118–127

Vinkovic T, Novák O, Strnad M, Goessler W, Jurašin DD, Paradikovic N, Vrček IV (2017) Cytokinin response in pepper plants (*Capsicum annuum* L.) exposed to silver nanoparticles. Environ Res 156:10–18

Wang J, Shu K, Zhang LI, Youbin S (2017) Effects of silver nanoparticles on soil microbial communities and bacterial nitrification in suburban vegetable soils. Pedosphere 27:482–490. https://doi.org/10.1016/S1002-0160(17)60344-8

Xie YK, Dong HR, Zeng GM, Tang L, Jiang Z, Zhang C, Deng JM, Zhang LH, Zhang Y (2017) The interactions between nanoscale zero-valent iron and microbes in the subsurface environment: a review. J Hazard Mater 321:390–407

Yausheva E, Sizova E, Lebedev S, Skalny A, Miroshnikov S, Plotnikov A, Khlopko Y, Gogoleva N, Cherkasov S (2016) Influence of zinc nanoparticles on survival of worms *Eisenia fetida* and taxonomic diversity of the gut microflora. Environ Sci Pollut Res 23(13):13245–13254

Yirsaw BD, Mayilswami S, Megharaj M, Chen Z, Naidu R (2016) Effect of zero valent iron nanoparticles to *Eisenia fetida* in three soil types. Environ Sci Pollut Res 23:9822–9831. https://doi.org/10.1007/s11356-016-6193-4

Zuverza-Mena N, Medina-Velo IA, Barrios AC, Tan W, Peralta-Videa JR, Gardea-Torresdey JL (2015) Copper nanoparticles/compounds impact agronomic and physiological parameters in cilantro (*Coriandrum sativum*). Environ Sci Process Impacts 17:1783–1793

Zuverza-Mena N, Martinez-Fernandez D, Du WC, Hernandez-Viezcas JA, Bonilla-Bird N, Lopez-Moreno ML, Komarek M, Peralta-Videa JR, Gardea-Torresdey JL (2017) Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses—a review. Plant Physiol Biochem 110:236–264

CONCLUSIONES GENERALES

La biosíntesis de NPs de cobre con la intervención de microalgas es posible. Esta técnica ofrece la posibilidad de controlar la calidad de estas en base a la cepa, el control de la iluminación y la salinidad durante el cultivo. La optimización del cultivo de determinada cepa para la producción de biomasa no es necesariamente la mejor opción para la producción de NPs de cobre. El cultivo con baja salinidad y alta iluminación de las tres cepas evaluadas ofrece las mejores posibilidades para la biosíntesis de NPs de cobre. De las tres cepas evaluadas, *T. suecica* mostró mayores posibilidades de éxito para la biosíntesis en diferentes condiciones de cultivo, mientras que *Ch. kessleri,* bajo condiciones autotróficas puede ser una buena opción para la síntesis de NPs de óxidos de cobre bajo diferentes condiciones de cultivo.

La producción de microalgas con la utilización de agua dulce representa un factor limitante por la gran cantidad de agua necesaria. Por otra parte, el uso de aguas residuales tiene un gran potencial para este fin. Existe la posibilidad de integrar el tratamiento de aguas residuales con la producción de biomasa para la producción de NPs metálicas. Sin embargo, aún falta más investigación y desarrollo en este sentido.

REFERENCIAS BIBLIOGRÁFICAS

Agrawal SC (2012) Factors controlling induction of reproduction in algae -review: the text. Folia Microbiol 57:387–407.

Alkhamis Y, Qin JG, (2013) Cultivation of *Isochrysis galbana* in phototrophic, heterotrophic, and mixotrophic conditions. BioMed Research International ID 983465, http://dx.doi.org/10.1155/2013/983465.

Barwal I, Ranjan P, Kateriya S, Yadav SC (2011) Cellular oxido-reductive proteins of *Chlamidomonas reinhardtii* contro the biosynthesis of silver nanoparticles. Journal of Nanobiotechnology 2011:9-56.

Behravan M, Panahi AH, Naghizadech A, Ziaee M, Mahdavi R, Mirzapour A (2019) Facile Green synthesis of silver nanoparticles using Berberis vulgaris leaf and root aqueous extract and its antibacterial activity. International Journal of Biological Macromolecules 124: 148-158.

Chang RL, Ghamsari L, Manichaikul A, Hom EF, Balaji S, Fu W, Shen Y, Hao T, Palsson BO, Salehi-Ashtiani K, Papin JA (2011) Metabolic network reconstruction of Chlamydomonas offers insight into light-driven algal metabolism. Mol Syst Biol 7:518. doi: 10.1038/msb.2011.52

Chen C-Y, Yeh K-L, Aisyah R, Lee D-J, Chang J-S (2011) Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour Technol 102:71–81.

Christenson L, Sims R (2011) Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol. Adv. 29:686–702.

Chaudhuri SK, Malodia L (2017) Biosynthesis of zinc oxide nanoparticles using leaf extract of Calotropis gigantean: characterization and its evaluation on tree seedling growth in nursery stage. Applied Nanosciences 7:501-512.

Cirulis J, Scott J, Ross G (2013) Management of oxidative stress by microalgae. Canadian Journal of Physiology and Pharmacology 19:15-21.

Craggs RJ, Lundquist T, Benemann J (2012) Wastewater treatment pond algal production for biofuel. En: The Science of Algal Fuels, Gordon R y Seckbach Eds. Springer Netherlands. p 425-445.

Dahoumane SA, Jeffryes C, Mechouet M, Agathos SN (2017) Biosynthesis of inorganic nanoparticles: A fresh look at the control of shape, size and composition. Bioengineering doi: 10.3390/bioengineering4010014

Dasgupta N, Ranjan S, Ramalingam C (2017) Applications of nanotechnology in agriculture and water quality management. Environ. Chem. Lett. 15:591-605.

Duhan JS, Kumar R, Kumar N, Kaur P, Nehra K Duhan S (2017) Nanotechnology: The new perspective in precision agriculture. Biotechnology Reports 15:11-23.

Dwivedi S (2012) Bioremediation of heavy metal by algae: Current and future perspective. Journal of Advanced Laboratory Research in Biology 195-199.

Gordon R, Seckbach J (Eds) (2012) The Science of Algal Fuels. Springer Dordrecht. ISBN 978-94-007-5110-1

He X, Deng H, Huang H-m (2019) The current application of nanotechnology in food and agriculture. Journal of Food and Drug Analysis 27:1-21.

Hii YS, Soo CL, Chuah TS, Mohd-Azmi A, Abol-Munafi B (2011) Interactive effect of ammonia and nitrate on the nitrogen uptake by *Nannochloropsis* sp. Journal of Sustainability Science and Management 6:60-68.

Hussain M, Raja NI, Mashwanil ZUR, Iqbal M, Sabir S, Yasmeen F (2017) In vitro seed germination and biochemical profiling of Artemisia absinthium exposed to various metallic nanoparticles. Biotech 7:101. Doi:10.1007/s13205-017-0741-6

Jena J, Pradhan N, Nayak RR, Dash BP, Sukla LB, Panda PK, Mishra BK (2014) Microalga *Scenedesmus* sp.: A potential low-cost green machine for silver nanoparticle synthesis. J. Microbiol. Biotechnol. 24:522-533.

Kaphle A, Navya PN, Umapathi A, Daima HK (2018) Nanomaterials for agriculture, food and environment: applications, toxicity and regulation.

Khot LR, Sankaran S, Maja JM, Ehsani R, Schuster EW (2012) Applications of nanomaterials in agricultural production and crop protection. Crop Protection 35:64-70.

Kim CW, Sung MG, Nam K, Moon M, Kwon J-H, Yang J-W (2014) Effect of monochromatic illumination on lipid accumulation of Nannochloropsis gaditana under continuous cultivation. Bioresour Technol 159:30–35.

Larayetan R, Ojemaye MO, Okoh OO, Okoh AL (2019) Silver nanoparticles mediated by *Callistemon citrinus* extracts and their antimalarial, antitrypanosoma and antibacterial efficacy. Journal of Molecular Liquids 273:615-625.

Li X, Xu H, Chen Z, Chen G (2011) Biosynthesis of Nanoparticles by Microorganisms and their applications. Journal of nanomaterials. doi:10.1155/2011/270974

Maanvizhi S, Nandhini S, Thangapandi P (2018) A green approach mediated synthesis of silver nanoparticles using extracts of Ananas comosus (Pineapple) waste: Characterization and antibacterial evaluation. Research Pharmaceutica 2:6-9. ISSN No: 2457-0389

Mohseniazar M, Barin M, Zarredar H, Alizadeh S, Shanehbandi D (2011) Potential of microalgae and lactobacilli in biosynthesis of silver nanoparticles. BioImpacts 1:149-152.

Montemezzani V, Duggan IC, Hogg ID, Braggs RJ (2017) Control of zooplankton populations in a wastewater treatment High Rate Algal Pond using overnight CO_2 asphyxiation. Algal Research 26:250-264.

Nayantara y Kaur P (2018) Biosythesis of nanoparticles using eco-friendly factories and their role in plant pathogenicity: a review. Biotechnology Research and Innovation 2:63-73.

Nicoletti M (2016) Microalgae nutraceuticals. Foods 5,54; doi:10.3390/foods5030054

Panyuta O, Belave V, Fomaidi S, Kalinichenko O, Volkogon M, Taran N (2016) The effect of pre-sowing seed treatment with metal nanoparticles on the formation of the defensive reaction of wheat seedlings infected with the eyespot causal agent. Nanoscale Research Letters 11:92 doi:10.1186/s11671-016-1305-0

Parisi C, Vigani M, Rodríquez-Cerezo E (2015) Agricultural nanotechnologies: What are the current possibilities? Nano today 10:124-127.

Patel V, Berthold D, Puranik P, Gantar M (2015) Screening of cyanobacteria and microalgae for their ability to synthesize silver nanoparticles with antibacterial activity. Biotechnology Reports 5:112-119.

Perales-Vela H, Peña-Castro J, Cañizares-Villanueva R (2006) Heavy metal detoxification in eukaryotic microalgae. Chemosphere doi: 10.1016/j.chemosphere.2005.11.024

Rubina MS, Yu A, Vasil`koy, Naumkin AV, Shtykova EV, Abramchuk SS, Alghuthaymi MA, Abd-Elsalam KA (2017) Synthesis and characterization of chitosan-copper nanocomposites and their fungicidal activity against two sclerotia-forming plant pathogenic fungi. J. Nanostruct Chem 7:249-258.

Ruttkay-Dedecky B, Krystofova O, Nejdl L, Adam V (2017), Nanoparticles based on essential metals and their phytotoxicity. Journal of Nanobiotechnology 15:33. doi:10.1186/s12951-017-0268-3

Sabatini SE, Juárez AB, Eppis MR, Bianchi L, Luquet CM, Ríos de Molina MC (2009) Oxidative stress and antioxidant defenses in two Green microalgae exposed to copper. Ecotoxicology and Environmental Safety 72:1200-1206.

Sekhon BS (2014) Nanotechnology in agri-food production: an overview. Nanotechnology, Science and Applications 7:31-53.

forza E, Simionato D, Giacometti GM, Bertucco A, Morosinotto T (2012) Adjusted light and dark cycles can optimize photosynthetic efficiency in algae growing in photobioreactors. PLoS One e38975. doi: 10.1371/journal.pone.0038975

Shah M, Fawcett D, Sharma S, Tripathy SK, Poinern GEJ (2015) Green synthesis of metallic nanoparticles via biological entities. Materials 8:7278-7308.

Sharmila G, Muthukumaran C, Sandiya K Santhiya S, Pradeep RS, Kumar NM, Suryanarayanan N, Thirumarimurugan M (2018) Biosynthesis, characterization, and antibacterial activity of zinc oxide nanoparticles derived from Bauhinia tormentosa leaf extract. Journal of Nanostructure in Chemistry 8:293-299.

Sheehan J, Dunahay T, Benemann J, Roessler P (1998) A look back at the U.S. Department of Energy´s aquatic species program: biodiesel from algae. Report by National Renewable Energy Laboratory, Colorado US. 291 p. NREL/TP-580-24190

Siddiqi K, Husen A (2016) Fabrication of metal and metal oxide nanoparticles by algae and their toxic effects. Nanoscale Research Letters 11:363. doi: 10.1186/s11671-016-1580-9

Sidhu A, Barmota H, Bala A (2017) Antifungal evaluation studies of copper sulfide nano-aquaformulations and its impact on seed quality. Applied Nanosciences 7:681-689.

Singh J, Dutta T, Kim KH, Rawat M, Samddar P, Kumar P (2018) ´Green´ synthesis of metals and their oxide nanoparticles: applications for environmental remediation. Journal of Nanobiotechnology 16:84. https://doi.org/10.1186/s12951-018-0408-4

Siriam S, Seenivasan R (2012) Microalgae cultivation in wastewater for nutrient removal. Journal of Algal Biomass Utilization 3:9-13.

Soleimani M, Habibi-Pirkoohi H (2017) Biosynthesis of silver nanoparticles using *Chlorella vulgaris* and evaluation of the antibacterial efficacy against *Staphylococcus aureus.* Avicenna Journal of Medical Biotechnology 9:120-125.

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Comercial applications of microalgae. Journal of Bioscience and Bioengineering 101; 87-96.

Sudha SS, Rajamanickam K, Rengaramanujam J (2013) Microalgae mediated synthesis of silver nanoparticles and their antibacterial activity against pathogenic bacteria. Indian Journal o Experimental Biology 52:393-399.

Taran N, Batsmanova L, Konotop Y, Okanenko A (2014) Redistribution of elements of metals in plant tissues under treatment by non-ionic colloidal solution of biogenic metal nanoparticles. Nanoscale Research Letters 9:534. http://www.nanosclereslett.com/content/9/1/354

Taran N, Storozhenko V, Svitlova N, Batsmanova L, Shvartau V, Kovalenko M (2017) Effect of Zinc and copper nanoparticles on drought resistance of wheat seedlings. Nanoscale Research Letters 12:60. Doi: 10.1186/s11671-017-1839-9

Ting H, Haifeng L, Shanshan M, Zhang Y, Zhidan L, Na D (2017) Progress in microalgae cultivation photobioreactors and applications in wastewater treatment: A review. Int. J. Agric. Biol. Eng. 10:1–29. doi:10.3965/j.ijabe.20171001.2705

Wintachai P, Paosen S, Yupanqui CT, Vorabunthikunchai SP (2019) Silver nanoparticles synthesized with *Eucalyptus critriodora* ethanol leaf extract stimulate antibacterial activity against clinically multidrug-resistant *Acinetobacter baumanni* isolated from pneumonia patients. Microbial Pathogenesis 126:245-257.

Worral EA, Hamid A, Mody KT, Mitter N, Pappu HR (2018) Nanotechnology for plant disease management. Agronomy 8,285; doi:10.3390/agronomy8120285

Yu W, Ansari W, Scoepp NG, Hannon MJ, Mayfield SP, Burkart MD (2011) Modification of the metabolic pathways of lipid and triacylglycerol production in microalgae. Microbial Cell Factories. 10: 11 p.