# IMPACT OF ENGINEERED NANOPARTICLES ON VIRULENCE OF *XANTHOMONAS ORYZAE* PV *ORYZAE* AND ON RICE SENSITIVITY AT ITS INFECTION

# IMPATTO DELLE NANOPARTICELLE INGEGNERIZZATE SULLA VIRULENZA DI *XANTHOMONAS ORYZAE* PV *ORYZAE* E SULLA SENSIBILITÀ DEL RISO ALLA SUA INFEZIONE

# IMPACT DE NANOPARTICULES DE SYNTHÈSE SUR LA VIRULENCE DE *XANTHOMONAS ORYZAE* PV *ORYZAE* ET SUR LA SENSIBILITÉ DU RIZ A SA INFECTION

**Giuliano Degrassi [(1)], Livia Vittori Antisari [(2)] *, Vittorio Venturi [(1)], Serena Carbone [(2)], Antonietta M. Gatti [(3)], Chiara Gambardella [(4)], Carla Falugi [(4)], Gilmo Vianello [(2)]**

[(1)] International Centre for Genetic Engineering and Biotechnology, Trieste, Italy
[(2)] Department of Agricultural Sciences, University of Bologna, Italy
[(3)] Nanodiagnostics srl, Modena, Italy
[(4)] Department of Earth, Environmental and Life Sciences, University of Genova, Italy

* Corresponding author: e-mail livia.vittori@unibo.it

**Abstract**
The present work of nanoecotoxicity wants to propose a new plant model starting from the rice plant. The model takes into consideration the impact of engineered nanoparticles (Ag, Co, Ni, $CeO_2$, $Fe_3O_4$, $TiO_2$) on rice plants that were weakened by infections of *Xanthomonas oryzae* pv *oryzae* bacteria. The results indicate that some NPs increase the rice sensitivity to the pathogen while others decrease the virulence of the pathogen towards rice. No-enrichment in component metal concentration is detected in above organs of rice, with exception of Ni-NPs treatment. An imbalance of major elements in infected rice crops treated with NPs was investigated.
**Keyworks:** *engineered nanoparticles, Ag, Co, Ni, $CeO_2$, rice, Xanthomonas oryzae*

## Introduction

Nanoparticles (NPs) are entities that can be either of natural origin, or generated by engineering processes or unintentionally produced by combustion and NPs will find their way into aquatic, terrestrial and atmospheric environment (Dinesh et al., 2010). Nanotechnology is currently an area of intense scientific interest due to a wide variety of potential applications of this products in biomedical, optical and electronic fields, but could also present possible negative effects from the medical

DOI: 10.6092/issn.2281-4485/4556

and environmental point of view. Indeed, most of these effects are related to the high surface to volume ratio, which can make the particles very reactive or catalytic due to their size, namely from 1 to100 nm. They can pass through cell membranes in organisms, and their interactions with biological systems are relatively unknown. The importance of understanding the NPs impact on the environment is growing day by day due to the potential increase of the release into the environment and also because NPs are present in several products already on the market. The plants are essential base of environment and NPs will inevitably interact with plants, these interactions, such as uptake and accumulation in plant biomass, will greatly affect their fate and transport in the environment. NPs could exert physical and/or chemical toxicity on plants (Ma et al, 2011). The interaction between NPs and plants were object of studies (Lin et al., 2007), focusing the potential toxicity and both positive and negative or negligible effects have been described (Menard et al., 2006). Evidence that NPs penetrate into plant cell were also reported with or without showing adverse effects (Khodakovskaya et al., 2009; Birbaum et al., 2010; Cifuentes et al., 2010).

In this work, we propose a complex biological systems represented by rice crop and *Xanthomonas oryzae* pv *oryzae,* its pathogen, to study the effects of NPs on crop, pathogen and the interaction between crop-pathogen. The rice represents the staple food for more than two billion people every day. There are many bacteria associated to rice, some of these are pathogenic and cause severe damages to the crop, such as *Xanthomonas oryzae* pv *oryzae* (Shen and Ronald, 2002), *Burkholderia glumae* and *B. plantarii* (Cottyn et al., 1996; Coenye et al., 1999) and *Pseudomonas fuscovaginae* (Mattiuzzo et al., 2011). In particular, *Xanthomonas oryzae* pv *oryzae* (*Xoo*) cause bacterial blight one of the most destructive rice disease, resulting in 10% to 80% yield losses, endangering worldwide food security (Zhu et al., 2000). Protection of crop plants from bacterial disease can substantially improve agricultural production. Synthetic or fumigant chemicals including inorganic, organic and metallic compounds are agents controlling the crop diseases with advise impact on the environment. The alternative is the resistance of rice by means of new transgenic technique. Recently, engineered NPs metal-based were tested as antimicrobial agents; they entry in cells of many bacteria (e.g. *Escherichia coli*, *Pseudomonas* ). The graphene oxide exhibits a high efficiency to inactive the bacteria pathogen of rise (Chen et al., 2013).

With these considerations, the aim of this study was to evaluate: 1) the efficiency of *Xanthomonas oryzae* pv *oryzae (Xoo)* exposed to metal (Ag, Co, Ni) and metal oxides ($CeO_2$, $Fe_3O_4$, $TiO_2$) nanoparticles infecting rice crop; 2) the effects of NPs on rice crop infected by *Xoo* after their addition to soil, mimicking the NPs contamination due to polluted biosolid or wastewater.

Images using electron microscopy (ESEM-EDX) were carried out to evaluate the pathogens culture, while the assessment of the uptake and translocation of NPs in crops was determined using inductive coupled plasma spectrometry (ICP-OES) after acidic mineralization of different organs (root, stem, panicles/seeds).

## Materials and methods

### Bacterial strain and growth conditions

The bacterial strain used in this study is *Xanthomonas oryzae* pv. *oryzae* XKK.12 (*Xoo*), isolated in Kerala State (India) and previously reported as being highly virulent to rice (Ferluga *et al.*, 2007). *Xoo* was grown at 28°C on peptone-yeast extract liquid medium (PY; 0,8% peptone, 0.2% yeast extract, 0,2% $K_2HPO_4$, 0,05% $KH_2PO_4$, 0,025% $MgSO_4$ $7H_2O$, 0.5% glucose w/v) or on peptone-sucrose agar plates (peptone 1%, sucrose 1%, agar 1,5% w/v). *Xoo* was grown in PY medium with and without NPs at concentration of 100 μg $mL^{-1}$.

### Rice growth

The plant used for the study of host-bacteria interaction is *Oryza sativa* Italian cultivar Baldo. Rice was grown either in a greenhouse (without control of humidity and light, temperature kept below 32ºC) for $CeO_2$, Co, $TiO_2$, Ni -NPs treatments and in a phytotron (with temperature of 27ºC, relative humidity of 50% and a light-dark photoperiod of 16:8) for Ag-NP treatment. Rice was fertilized with 10 $g/m^2$ of iron sulfate and 5 $g/m^2$ of ammonium sulphate every two weeks, and daily watered. For this reason we present the data separated for the two different growth conditions.

### Nanoparticles

The plants were exposed to: magnetite ($Fe_3O_4$; 15-20 nm), passivated cobalt (Co, 28 nm), titanium oxide ($TiO_2$; 20-160 nm), nickel (Ni, 62 nm), silicon oxide ($SiO_2$, 4-40 nm), cerium oxide ($CeO_2$, 50-105 nm), tin oxide ($SnO_2$, 61 nm). All the chemicals and reagents were purchased from Sigma Aldrich (Italy), unless otherwise stated. $SnO_2$, $CeO_2$ and $Fe_3O_4$ NPs were provided by Nanostructured & Amorphous Materials, Inc (Houston, USA) with at least 98% purity; $SiO_2$ was provided by Tal Materials Inc (Michigan, USA) with at least 98% purity. By manufacturer's information the specific surface area was 14.2, 8-15, > 40 m and 400 $m^2/g$ and the particle size, calculated from the specific surface area and by Transmission Electron Microscopy was 61, 50-105, 20-30, 4-40 nm for $SnO_2$, $CeO_2$ $Fe_3O_4$ and $SiO_2$, respectively. Stocks of NPs at concentration of 10 mg $mL^{-1}$ were used and always sonicated before the appropriate dilution.

### Experimental design

To assess the influence of NPs on the virulence of *Xanthomonas oryzae* pv *oryzae* (*Xoo*), rice leaves were infected by means of the clipping method previously reported by Ferluga *et al*., (2007). Briefly, 45 to 60 days old rice leaves, grown in greenhouse and phytotron, were infected with overnight cultures of *Xoo* following the schema in which the control was the rice infected by *Xoo* (R/*Xoo*) and the

DOI: 10.6092/issn.2281-4485/4556

treatments were: i) rice+NP infected by *Xoo* (R-NP/*Xoo*), ii) rice infected by *Xoo*-NP treated (R/*Xoo*-NP) and iii) rice+NP infected by *Xoo*+NP (R-NP/*Xoo*-NP). The NPs were added to soil at 500 µg $kg^{-1}$ of dry weight, while the pathogens were grown in pure culture with NPs at concentration of 100 µg $mL^{-1}$.
Optical density $OD_{600}$ of the overnight cultures of *Xoo* grown with NPs was measured and the cell number diluted to $1x10^9$ $mL^{-1}$. These diluted cultures were used to inoculate rice leaves, 3 to 4 leaves per plant, ten plants for each treatment combination (R/*Xoo*-NP and R-NP/*Xoo*-NP) were tested. Scissors were dipped into the cultures and then used to cut the tip of rice leaves. Plants were then incubated for 24 hours in a humidity chamber (27ºC and more than 95% of humidity), then moved to the greenhouse. Virulence and symptoms were measured after two weeks by measuring the lesion lengths caused by the pathogens along the central vein (Figure 1). A preliminary experiment compared all the treatments, while a second experiment was performed limited to Co-, $CeO_2$-, and $TiO_2$-NPs, which showed to enhance the bacteria virulence.

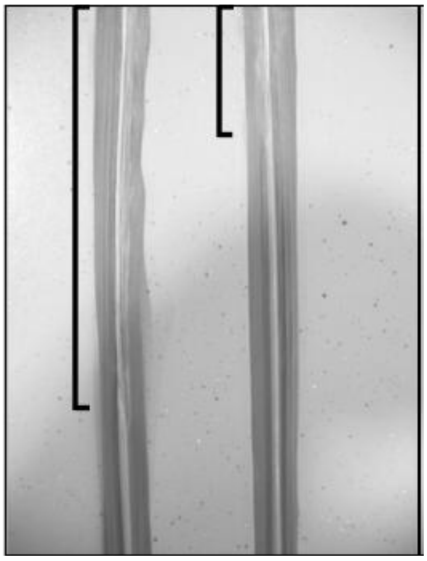

**Figure 1**
*Lesion along the central vein on leaves inoculated with Xanthomonas oryzae pv oryzae by the clipping method. The measure of the lesions in cm is the parameter of the bacteria virulence*

Plants were grown in trays (50x40x9cm) as twenty plants per tray, one tray for each NP tested. Rice plants were grown up to the completion of the flowering stage and seeds production and they were harvested and analysed for: i) average number of panicles per plant, ii) number of seeds containing starch ("full seeds" vs "empty seeds"); iii) percentage of germination of "full seeds" (seeds were germinated in microtitre plates, one seed in one well with the addition of 100 mL of sterile water). In addition seeds were tested for the germination ability under standard germination condition (in Petri dishes, onto a water-soaked filter paper, at 28ºC for four days).
At the end of the rice growth cycle ten rice plants were sampled and divided in root, stem and inflorescences. The rice tissues were dried in a forced air oven

(T<40°C) and homogenized (in a blender with blades made of pure titanium, carefully checked to not introduce any further metal contamination to samples). Unwashed plant samples were used. The mineralization of rice tissues was carried out according to Vittori Antisari et al., (2011). Briefly, 0.25 g sub-sample of plant tissues was dissolved in 8 mL of concentrated $HNO_3$ (suprapure, Meck) plus 2 mL of $H_2O_2$ (Carlo Erba for electronic use). The mineralization was carried out in Teflon bombs in a microwave oven (Milestone, 1200). After cooling, solutions were made up to 20 mL with distilled water (Milli-Q, Millipore) and then filtered with Whatman 42. The accuracy of the instrumental method and analytical procedures used was checked using reference material, which was run after every 10 samples to check for drift in the sensitivity. The reference materials, provided by the European Commission Institute for Reference Materials and Measurements, were: CRM 060 (aquatic plants), CRM 062 (Olive leaves). The solution, obtained after mineralisation for both samples and reference materials, were analysed by Inductive Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Spectro Ametek, Arcos).
Statistical analysis of data was performed by one-way ANOVAs analysis of variance with post-hoc Turkey's multiple comparison test.

## Results

**Greenhouse experiments: Influence of NPs treatments on rice-*Xoo* system.**
After 14 days from infection with *Xanthomonas oryzae* pv *oryzae* (*Xoo*) the length of lesions (in cm) was analysed and the treatments with Co-, $CeO_2$-, and $TiO_2$-NPs were more virulent than the control (R/Xoo), as well as the preventive treatment of *Xoo* with Co-NPs (R/Xoo-Co) showed a lower lesion length than the control. The rice plants contaminated with Ni- and $Fe_3O_4$- NPs showed the same behaviour and no significant differences with respect to the control was observed (data no shown). The exposure of the rice plants at Co-, $CeO_2$- and $TiO_2$-NPs, added into soil through irrigation water, showed more virulent disease and the crop seemed to be more sensitive to *Xoo* infection. These results induced to plan new experiments testing again Co-, $CeO_2$ and $TiO_2$-NPs. One-way ANOVA test was performed to underline statistically differences among the different treatments and the results for $CeO_2$-, Co-, and $TiO_2$-NPs treatments were reported in Table 1.

**Interaction between *Xanthomonas oryzae* pv *oryzae* and NPs using ESEM-EDX.**
The interaction between *Xoo* and NPs was detected by means of electron microscopy (ESEM-EDX); Figure 1 shows the pathogen grown in PY medium without NPs, while in Figure 2 and 3 two examples of *Xoo* grown in the presence of $Fe_3O_4$- and Co-NP, respectively, were reported.

DOI: 10.6092/issn.2281-4485/4556

**Table 1.** *Using One-way ANOVA analysis of variance with post-hoc Turkey's (p<0.05) multiple comparison test of Figure 2 (Ti, Ce and Co only)*

| **Rice+$TiO_2$-NP** | Mean Diff. | q | P < 0.05 | 95% CI of diff |
|---|---|---|---|---|
| Rice+Ti/Xoo+Ti *vs* Rice+Ti/Xoo | -2.1 | 2.5 | ns | -5.0 to 0.75 |
| ice+Ti/Xoo+Ti *vs* Rice/Xoo | 6.6 | 9.0 | *** | 4.1to 9.1 |
| Rice+Ti/Xoo *vs* Rice/Xoo | 8.8 | 11.7 | *** | 6.2 to 11.3 |
| **Rice +$CeO_2$-NP** | Mean Diff. | q | P < 0.05 | 95% CI of diff |
| Rice+Ce/Xoo+Si *vs* Rice+Ce/Xoo | 1.0 | 1.3 | ns | -1.5 to 3.5 |
| Rice+Ce/Xoo+Si *vs* Rice/Xoo | 6.3 | 9.7 | *** | 4.1 to 8.4 |
| Rice+Ce/Xoo *vs* Rice/Xoo | 5.3 | 8.2 | *** | 3.1 to 7.4 |
| **Rice +Co-NP** | Mean Diff. | q | P < 0.05 | 95% CI of diff |
| Rice+Co/ Xoo+Co *vs* Rice+Co/Xoo | -0.4596 | 0.5605 | ns | -3.5 to 2.6 |
| Rice+Co/Xoo+Co *vs* Rice/Xoo | 2.9 | 4.3 | * | 0.4 to 5.4 |
| Rice+Co/Xoo+Co *vs* Rice/Xoo+Co | 6.1 | 9.0 | *** | 3.6 to 8.6 |
| Rice+Co/Xoo *vs* Rice/Xoo | 3.4 | 4.8 | ** | 0.8 to 5.9 |
| Rice+Co/Xoo *vs* Rice/Xoo+Co | 6.6 | 9.3 | *** | 3.9 to 9.2 |
| Rice/Xoo*vs* Rice/Xoo+Co | 3.2 | 6.1 | *** | 1.3 to 5.2 |

The results obtained confirmed that *Xoo* grown in laboratory media in presence of Co-NPs was less virulent in the rice plant. In addition the rice plant displays slightly higher sensitivity to *Xoo* infection when they are grown after addition of Co-NPs. $CeO_2$- and $TiO_2$-NPs into soil inducing significantly ($p<0.05$) higher pathogen virulence than the control one.

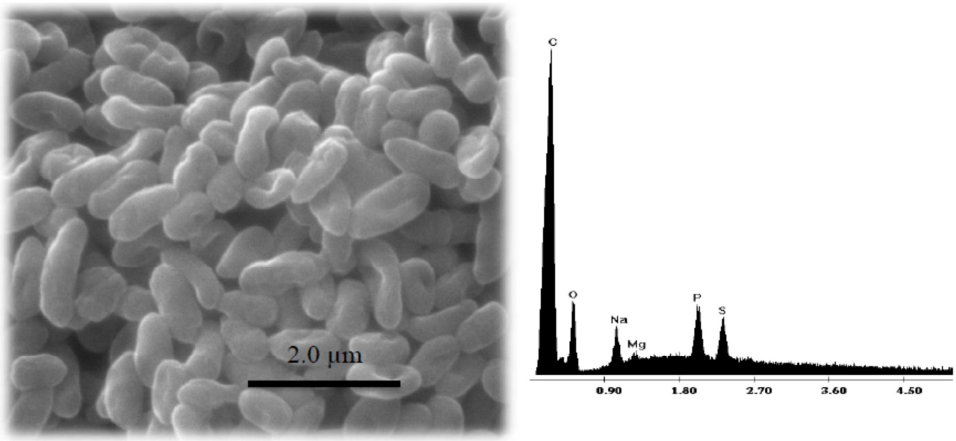


**Figure 1**. *Xanthomonas oryzae pv oryzae cells grown in PY medium without NPs and observed by electron microscopy (ESEM-EDX)*

The electron microscope images showed that the magnetite (Figure 2) seemed to form a cluster and aggregates of nanoparticles on the cells of *Xoo*. The Co-NP

showed little aggregates of smaller sizes, isolated particles (Figure 3), also the presence of Co-NP seemed to released organic substances by cells (Figure 3).

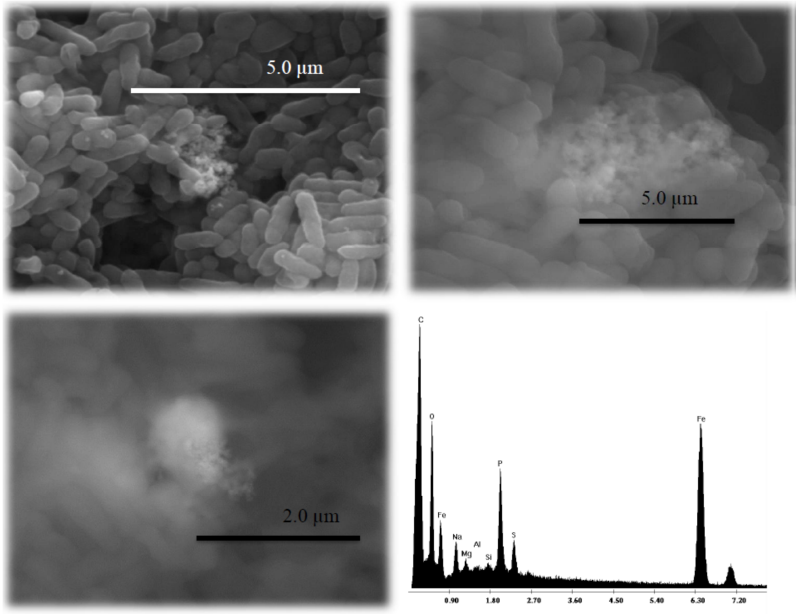


**Figure 2**. *Xanthomonas oryzae* pv *oryzae cells grown in PY medium with Fe and observed under scanning electron microscopy (ESEM-EDX)*

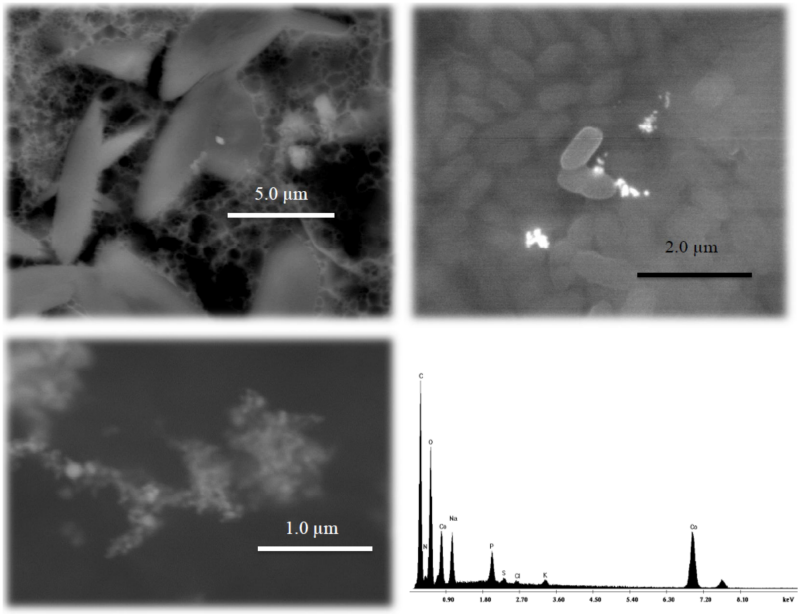


**Figure 3.** *Xanthomonas oryzae* pv *oryzae cells grown in PY medium with Co and observed under scanning electron microscope (ESEM-EDX)*

**Panicle and seed formation and fertility.** The rice plants overcome the pathogen infection were grown up to the seeds formation; the number of both panicles and seeds were counted (Table 2). No significant differences were found between the treatments and the control, even if, as expected, the rice no-exposed at *Xoo* and at

DOI: 10.6092/issn.2281-4485/4556

NPs showed high parameters, the contrary for the crops infected by the pathogens. The calculation of germination percentage was performed with "full seeds" and no significant differences in germination rate were observed in among the different seed batches/samples (Table 2).

Although the statistical elaboration of data (ANOVA) did not show significant results, nevertheless a strong trend was observed for the effects exerted by the exposure to the different NPs. Thus, we calculated the relative risk (RR) showing the probability of the effect occurring in the exposed group versus a non-exposed group. (Sistrom and Garvan, 2004)

$$\boldsymbol{RR} = \frac{\boldsymbol{P} \text{ event when exposed}}{\boldsymbol{P} \text{ event when non-exposed}}$$

Generally, on the basis of this statistical approach the probability of negative effect on seed germination arising from NPs exposure increased than the control ones; about 2.5 times greater in the plants exposed to Ni-NP than the control. The incidence of exposure to NPs was relevant mainly for the exposure of rice plants at NPs. In fact the presence of Ni-, Co- and $Fe_3O_4$- NPs could decrease the germination of the seed on rice plants contaminated. At the same time the presence of Co-NP (R+Co/Xoo and R+Co/Xoo-Co) in infected plants could ameliorate the germination phenomenon.

**Table2.** *Number panicles/rice plants, full seed and germination percentage are reported.*

| | Number panicles/plant | | Full seed | | Germination | |
|---|---|---|---|---|---|---|
| | | SD | % | SD | % | SD |
| R | 2.7 | 0.7 | 88.1 | 2.1 | 89.0 | 6.5 |
| R/Xoo | 1.8 | 1.1 | 76.4 | 0.5 | 81.2 | 3.5 |
| R/Xoo-Co | 2.6 | 0.5 | 86.6 | 0.6 | 83.7 | 4.0 |
| R+Ce | 1.8 | 0.8 | 79.4 | 0.3 | 84.2 | 7.4 |
| R+Ce/Xoo | 1.8 | 1.0 | 79.4 | 0.2 | 82.0 | 9.5 |
| R+Ce/Xoo-Ce | 1.8 | 0.7 | 87.1 | 0.2 | 86.3 | 5.5 |
| R+Co | 1.4 | 0.6 | 72.1 | 0.4 | 87.3 | 3.4 |
| R+Co/Xoo | 1.7 | 0.7 | 72.1 | 0.1 | 88.3 | 4.5 |
| R+Co/Xoo-Co | 1.2 | 0.4 | 85.1 | 0.8 | 86.3 | 2.5 |
| R+Ni | 1.4 | 0.6 | 84.7 | 1.0 | 78.0 | 8.0 |
| R+Ni/Xoo | 1.3 | 0.7 | 85.4 | 0.2 | 75.7 | 8.4 |
| R+Ni/Xoo-Ni | 1.5 | 0.5 | 84.0 | 0.4 | 80.3 | 8.6 |
| R+Fe3O4 | 1.7 | 0.7 | 81.7 | 0.8 | 81.2 | 6.9 |
| R+Fe/Xoo | 1.8 | 0.7 | 85.8 | 0.6 | 80.3 | 9.0 |
| R+Fe/Xoo-Fe | 1.6 | 0.7 | 77.6 | 0.5 | 82.0 | 6.1 |
| R+$TiO_2$ | 1.4 | 0.6 | 79.4 | 0.9 | 83.0 | 4.2 |
| R+Ti/Xoo | 1.4 | 0.7 | 78.1 | 0.6 | 83.7 | 4.0 |
| R+Ti/Xoo-Ti | 1.4 | 0.5 | 79.4 | 0.7 | 82.3 | 5.1 |

**Total concentration of elements arising NPs in rice crops**

Component metal concentration (e.g. Ce, Co, Ni, Fe, Ti) arising from NPs in rice crops organs is shown in Table 3. The ANOVA test confirmed that the

concentration of Co, Ce and Ni in roots was statistically higher ($p<0.05$) than the respective controls (R/Xoo and R/Xoo-Co), while no differences were found for $Fe_3O_4$- and $TiO_2$-NPs treatments (Table 3).
In both control and NP-treatment, Ce and Co concentration in epigeous organs was always lower than detection limits (DL) The storage of Fe in rice plants treated with magnetite and infected by both *Xoo* and *Xoo*-$Fe_3O_4$ was lower in roots and stem ($p<0.01$) than those of respective controls, while no significant difference was found in seeds. In $TiO_2$-NP treatments, the Ti concentration in stems was significant higher ($p<0.01$) than the controls, while no difference was found for the other organs. The Ni amount was detected in stem in rice plant exposed at Ni-NPs, the Ni concentration was also significantly higher in roots ($p<0.05$ and 0.01, respectively) in both rice treatments with Ni-NP added to soil.

| | **Ce** | **Co** | **Fe** | **Ni** | **Ti** |
|---|---|---|---|---|---|
| | | | µg $g^{-1}$ | | |
| **Panicles** | | | | | |
| R-NP/Xoo | DL | DL | 33.7 | DL | 2.1 |
| R/Xoo | DL | DL | 31.2 | DL | 2.6 |
| **ANOVA** | | | ns | | ns |
| R-NP/Xoo-NP | DL | DL | 21.9 | DL | 1.7 |
| R/Xoo-Co | DL | DL | 25.9 | DL | 1.6 |
| **ANOVA** | | | ns | | ns |
| **Stem** | | | | | |
| R-NP/Xoo | DL | DL | 47.8 | DL | 2.7 |
| R/Xoo | DL | DL | 62.1 | DL | 0.9 |
| **ANOVA** | | | ** | | ** |
| R-NP/Xoo-NP | DL | DL | 42.7 | 0.9 | 2.7 |
| R/Xoo-Co | DL | DL | 75.5 | DL | 0.9 |
| **ANOVA** | | | ** | ** | ** |
| **Root** | | | | | |
| R-NP/Xoo | 2.3 | 5.9 | 1757 | 3.4 | 31.3 |
| R/Xoo | DL | 0.5 | 2307 | 1.2 | 29.3 |
| **ANOVA** | ** | ** | ns | * | ns |
| R-NP/Xoo-NP | 3.1 | 5.2 | 1403 | 11.2 | 24.3 |
| R/Xoo-Co | DL | 0.6 | 1787 | 1.6 | 20.9 |
| **ANOVA** | ** | ** | * | ** | ns |

**Table 3**
*Total concentration elements arising from nanoparticles in rice organs.*
*ANOVA one-way is performed using Tukey test ($p<0.05$)*

## Distribution of major elements in rice plant organs

The rice plants harvested at the end of the crop cycle were analysed for major elements; in Table 4 are shown the data obtained by rice treated with NPs and inoculated with *Xoo*.
Generally, the Ca and Na concentration in panicles of rice plants treated with NPs were lower ($p<0.05$) than those of control. The major elements concentration in stem varied as a function of NPs treatments without a visible trend. We observed a very low concentration of K and P in the stem of R-Ni/*Xoo* treatment, as well as a

DOI: 10.6092/issn.2281-4485/4556

very high concentration of both of these nutrients in roots. Low P concentration was detected in roots treated with Co- and $Fe_3O_4$-NPs/*Xoo*.
The major nutrients concentration was not different between the rice plants treated with NPs and the pathogens incubated with NPs and the control in panicles (Table 4 - Continuation), with some exception, the values in stems were lower in rice treated than the control. In particular, the treatments with $CeO_2$ and Ni-NPs showed a lack of K accumulation in rice stems (Table 4). Ni-NP treatment showed high nutrients concentration in roots, for the other treatments no trend were observed.

**Table 4.** *Major elements in rice plant organs. The bold number are significantly different with respect to the control.*

| | **Ca** | **K** | **Mg** | **Na** | **P** | **S** |
|---|---|---|---|---|---|---|
| | mg $kg^{-1}$ | | | | | |
| **Panicles** | | | | | | |
| R-Co/Xoo | 664 | 4579 | 977 | **299** | **2322** | **874** |
| R-$CeO_2$/Xoo | **826** | 3907 | 1113 | **246** | **2243** | **898** |
| R-$Fe_3O_4$/Xoo | **594** | 4495 | **945** | **343** | 1971 | 937 |
| R-Ni/Xoo | **594** | 4495 | **945** | **343** | 1971 | 937 |
| R-$TiO_2$/Xoo | **589** | 4309 | **909** | **322** | 1973 | 913 |
| *Control (R/Xoo)* | *725* | *4410* | *1038* | *482* | *1896* | *1142* |
| **Stem** | | | | | | |
| R-Co/Xoo | **4829** | 3626 | 1827 | **1823** | **435** | **1579** |
| R-$CeO_2$/Xoo | **2974** | 2983 | **1721** | **2168** | 262 | **1573** |
| R-$Fe_3O_4$/Xoo | 3401 | **2744** | **1995** | **4206** | **710** | 2011 |
| R-Ni/Xoo | **3939** | **169** | **1724** | **5444** | **146** | **1489** |
| R-$TiO_2$/Xoo | **4772** | 2761 | **1995** | **2454** | **207** | 2023 |
| *Control (R/Xoo)* | *3661* | *3338* | *2808* | *3270* | *371* | *1966* |
| **Roots** | | | | | | |
| R-Co/Xoo | **5750** | **8117** | 1486 | **2886** | **582** | 1630 |
| R-$CeO_2$/Xoo | **4868** | **7460** | 1166 | **4728** | 1250 | 2528 |
| R-$Fe_3O_4$/Xoo | 6454 | 7074 | 1430 | **3716** | **821** | 1928 |
| R-Ni/Xoo | **10941** | **10415** | **3147** | **5451** | **3041** | 2857 |
| R-$TiO_2$/Xoo | 8526 | 7179 | 1490 | **3809** | 1458 | 2749 |
| *Control (R/Xoo)* | *9402* | *6741* | *1785* | *3052* | *1068* | *2861* |

## Discussion

The interaction between NPs and *Xanthomonas oryzae* pv *oryzae* was tested measuring the length of infection in the leaves (Niňo-Liu et al., 2006); Co-NP treatment shows the best efficiency in inhibiting the rice disease. For this reason, in all the experiments, the symptoms of control without NPs exposure (*Xoo*) is compared with *Xoo* grown in the presence of Co-NP. The culturing pathogens treated with Co-NP shows an interaction between the single nanoparticles and pathogens cells, while the interaction between the other NPs and the pathogen cells show cluster and agglomerations of nanoparticles. These later treatments do not

alter the *Xoo* virulence and pathogenicity. The exposure of rice crops at some NPs (Co-, $CeO_2$ and $TiO_2$) magnify the virulence of pathogen attack and the hypothesis can be due to a more vulnerability of crops exposed to NPs.

**Table 4.** *Continuation*

| | **Ca** | **K** | **Mg** | **Na** | **P** | **S** |
|---|---|---|---|---|---|---|
| | mg $kg^{-1}$ | | | | | |
| **Panicles** | | | | | | |
| R-Co/Xoo-Co | **539** | 4299 | 900 | **259** | **2217** | **824** |
| R-$CeO_2$/Xoo-$CeO_2$ | **609** | 4346 | 1043 | **291** | **2213** | **995** |
| R-$Fe_3O_4$/Xoo-$Fe_3O_4$ | **540** | **3941** | 1036 | **249** | **2100** | **881** |
| R-Ni/Xoo-Ni | **447** | 4411 | **849** | **288** | 1833 | 976 |
| R-$TiO_2$/Xoo-$TiO_2$ | **618** | 4835 | 1000 | **344** | **2190** | 980 |
| *Control (R/Xoo-Co)* | *715* | *4320* | *1025* | *478* | *1883* | *1005* |
| **Stem** | | | | | | |
| R-Co/Xoo-Co | 4495 | 3458 | **1771** | **1444** | 286 | **1532** |
| R-$CeO_2$/Xoo-$CeO_2$ | **2717** | **nd** | **1506** | **2749** | 315 | 1725 |
| R-$Fe_3O_4$/Xoo-$Fe_3O_4$ | **3067** | 5236 | **2209** | **2300** | 297 | **1355** |
| R-Ni/Xoo-Ni | **3763** | **nd** | **1644** | **5786** | **175** | **1472** |
| R-$TiO_2$/Xoo- $TiO_2$ | **2506** | **2290** | **1468** | **2833** | **548** | 1616 |
| *Control (R/Xoo-Co)* | *4350* | *4074* | *2877* | *3683* | *297* | *1804* |
| **Roots** | | | | | | |
| R-Co/Xoo-Co | 6905 | 6770 | 1542 | **2837** | 1041 | 2090 |
| R-$CeO_2$/Xoo-$CeO_2$ | **7754** | 7758 | 1454 | 3780 | **1808** | **2935** |
| R-$Fe_3O_4$/Xoo-$Fe_3O_4$ | 4937 | 7783 | 1349 | **4698** | 1153 | 2363 |
| R-Ni/Xoo-Ni | **9267** | **9003** | **2335** | **4488** | **2338** | **2740** |
| R-$TiO_2$/Xoo- $TiO_2$ | **7569** | 7585 | 1536 | **3510** | **1624** | 2610 |
| *Control (R/Xoo-Co)* | *7005* | *6773* | *1628* | *3840* | *1201* | *2598* |

Many discordant results are found in literature: about the interaction between nanoparticles and crops for examples $TiO_2$-NP showed no evidence of phytotoxicity in terms of germination and root elongation of tomatoes (Song et al., 2013); on the other hands, genotoxicity of $TiO_2$-NP was assessed *in vivo* in plants and *in vitro* in human lymphocytes (Ghosh et al., 2010).

Low translocation of nanoparticles in rice organs is found in this experiments, the infection with *Xoo* do not affect the NPs translocation. ICP-OES data show that the component metal concentration in above ground rice organs (e.g. leaves, stem) is low for both infection treatments. Different translocation behaviour for NPs is detected for other crops (Wang et al., 2012; Servin et al., 2012; 2013; Vittori Antisari et al., 2015).

Generally, little is known about the influence of NPs on plant mineral composition or on the macro- and microelement accumulation in different plant organs. In our case a decrease of Ca and Na concentration in panicles is not always balanced by an accumulation in the roots. In all cases, with exception for R-Ni/Xoo and R-Ni/Xoo-Ni, it appears that the plant will absorb a smaller amount of these elements from the soil. This phenomenon is associated with an increase of P in above organs

DOI: 10.6092/issn.2281-4485/4556

of rice. This evidence suggests a competitive inhibition of major nutrients due to NPs added into soils and also *Xoo* infection.

## Conclusions

The question of the uptake, bioaccumulation, biotransformation and risk of NPs in food crops is still not well understood.
The metal oxides and metallic engineered NPs were found to have differing effects on *Xanthomonas oryzae* pv *oryzae* and Co-NP inhibits the virulence of this important disease of rice.
The presence of nanoparticles in soil do not influence the disease of rice plants, exacerbating the symptoms. An imbalance of major elements in infected rice crops treated with NPs was observed.

**Acknowledgments** This study was supported by the INESE project funded by the Italian Institute of Technology (IIT, Genoa, Italy).

## References

BIRBAUM K., BROGIOLI R., SCHELLENBERG M., MARTINOIA E., STARK W.J., GÜNTHER D., *ET AL.* (2010) No evidence for cerium dioxide nanoparticle translocation in maize plants. Environmental Science & Technology, 44:8718-8723.
CIFUENTES Z., CUSTARDOY L., DE LA FUENTE J., MARQUINA C., IBARRA M.R., RUBIALES D. *ET AL.* (2010) Absorption and translocation to the aerial part of magnetic carbon-coated nanoparticles through the root of different crop plants. Journal of Nanobiotechnology, 8:26-34.
CHEN J., WANG X., HAN H. (2013) A new function of graphene oxide emerges: Inactivating phytopathogenic bacterium Xanthomonas oryzae pv. Oryzae. J. Nanopart. Res., 15:1658-1672. DOI: 10.1007/s11051-013-1658-6
COENYE T., HOLMES B., KERSTERS K., GOVAN J.R., VANDAMME P. (1999) Burkholderia cocovenenans (van Damme et al. 1960) Gillis et al. 1995 and Burkholderia vandii Urakami et al. 1994 are junior synonyms of Burkholderia gladioli (Severini 1913) Yabuuchi et al. 1993 and Burkholderia plantarii (Azegami et al. 1987) Urakami et al. 1994, respectively. Int J Syst Bacteriol., 1:37-42.
COTTYN B., VAN OUTRYVE M.F., CEREZ M.T., DE CLEENE M., SWINGS J., MEW T.W. (1996) Bacterial diseases of rice. II. Characterization of pathogenic bacteria associated with sheath rot complex and grain discoloration of rice in the Philippines. Plant Dis., 80:438-445.
DINESH R, ANANDARAJ M, SRINIVASAN V, HAMZA S (2012) Engineered nanoparticles in the soil and their potential implications to microbial activity. Geoderma 173–174:19–27. doi:10.1016/j.geoderma.2011. 12.018
FERLUGA S, BIGIRIMANA J, HÖFTE M, VENTURI V. (2007) A LuxR homologue of Xanthomonas oryzae pv. oryzae is required for optimal rice virulence. Mol Plant Pathol. 8:529-538. doi: 10.1111/j.1364-3703.2007.00415.x.

GHOSH M., BANDYOPADHYAY M., MUKHERJEE A. (2010) Genotoxicity of titanium dioxide ($TiO_2$) nanoparticles at two trophic levels: Plant and human lymphocytes. Chemosphere, 81:1253–1262.

LIN D., XING B. (2007) Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Environ Pollut. 150:243–250.

KHODAKOVSKAYA M., E. DERVISHI, M. MAHMOOD, Y. XU, Z. LI, F. WATANABE *et al.,* "Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth", ACS Nano. 3, pp: 3221-3227, 2009.

NIŇO-LIU D.O., RONALD P.C., BOGDANOVE A.J., 2006 Xantomonas oryzae pathovars: model pathogens of a model crop. Molecular Plant Pathology 7, 303-324.

MA X., J. GEISER-LEE, Y. DENG, A. KOLMAKOV (2011) "Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation", Science of The Total Environment, 408, 3053-3061

MATTIUZZO M, BERTANI I, FERLUGA S, CABRIO L, BIGIRIMANA J, GUARNACCIA C, PONGOR S, MARAITE H, VENTURI V. (2011) The plant pathogen Pseudomonas fuscovaginae contains two conserved quorum sensing systems involved in virulence and negatively regulated by RsaL and the novel regulator RsaM. Environ. Microbiol., 13:145-62.

SERVIN A.D., CASTILLO-MICHEL H., HERNANDEZ-VIEZCAS J.A., DIAZ B.C., PERALTAVIDEA J.R., GARDEA-TORRESDEY J.L. (2012) Synchrotron micro-XRF and micro-XANES confirmation of the uptake and translocation of $TiO_2$ nanoparticles in cucumber (Cucumis sativus) plants. Environ. Sci. Technol., 46:7637–7643. DOI:10.1021/ es300955b.

SERVIN A.D., MORALES M.I., CASTILLO-MICHEL H., HERNANDEZ-VIEZCAS J.A., MUNOZ B., ZHAO L., NUNEZ J.E., PERALTA-VIDEA J.R., GARDEA-TORRESDEY J.L. (2013) Synchrotron verification of TiO2 accumulation in cucumber fruit: a possible pathway of TiO2 nanoparticle transfer from soil into the food chain. Environ. Sci. Technol., 47:11592–11598. DOI:10.1021/es403368j

SHEN Y., RONALD P. (2002) Molecular determinants of disease and resistance in interactions of *Xanthomonas oryzae* pv. *oryzae* and rice. Microbes Infect., 4:1361-1367.

SISTROM C.L,, GARVAN C.W. (2004) Proportions, Odds, And Risk. Radiology, 230 (1):12–9.

SONG U., JUN H., WALDMAN B., ROH J., KIM Y., YI J., LEE E.J. (2013) Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO2 and Ag on tomatoes (*Lycopersicon esculentum*). Ecotoxicol. Environ. Saf., 93:60–67. DOI:10.1016/j. ecoenv.2013.03.033

VITTORI ANTISARI L., CARBONE S., FABRIZI A., GATTI A., VIANELLO G. (2011). Response of soil microbial biomass to $CeO_2$ nanoparticles. EQA, 7:135-150.

VITTORI ANTISARI L., CARBONE S., GATTI A., VIANELLO G., NANNIPIERI P. (2015) Uptake and translocation of metals and nutrients in tomato grown in soil polluted with metal oxide ($CeO_2$, $Fe_3O_4$, $SnO_2$, $TiO_2$) or metallic (Ag, Co, Ni) engineered nanoparticles. Environmental Science and Pollution. DOI 10.1007/s11356-014-3509-0

ZHU Y., CHEN H., FAN J., WANG Y., LI Y., *ET AL.* (2000) Genetic diversity and disease control in rice. Nature, 406:718–722

WANG Q., MA X., ZHANG W., PEI H., CHEN Y. (2012) The impact of cerium oxide nanoparticles on tomato (*Solanum lycopersicum* L.) and its implications for food safety. Metallomics, 4:1105–1112. DOI:10. 1039/c2mt20149f

DOI: 10.6092/issn.2281-4485/4556