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RESEARCH ARTICLE

# Evaluation of heavy metals, cytotoxicity, and antioxidant activity of tomatoes grown in toxic muddy soils

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**Abstract** This research studies tomatoes grown in polluted soils to ascertain their phytochemical and nutritive features. Pulp and seeds from tomatoes grown in muddy soils were analyzed for their antioxidant power and their toxicity because of the possibility that heavy metals were present in the soils. An antioxidant assay on methanol extracts was made by using DDPH, while an ABTS [2,2′-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)] assay was used to evaluate the antioxidant activity of lipophilic fractions. Results of the antioxidant assay showed that the tomatoes maintained a high level of antioxidant activity especially in the lipophilic fractions which contain the most representative compounds. Cytotoxic activity was performed on HeLa, PDAC, and A375 cell lines by [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2H-tetrazolium bromide] (MTT) assay. Results showed that neither the seeds, nor the pulp, of the extracts was cytotoxic. The presence of heavy metals was evaluated by using spectroscopy of atomic absorption with a graphite oven. Test results show the absence of heavy metals and these results have an interesting scientific role because they provide useful information for promoting food safety.

**Keywords** Antioxidant activity · Toxic muddy soil · Tomato · Heavy metals · Food safety · MTT assay

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## Introduction

Today, soil contamination is one of the most important environmental problems because of the serious risk it brings to the food supply. Among contaminants, heavy metals represent a major class of toxic agents in organisms because of the increasing use of them by industry, which results in high bioaccumulation (Verkleij et al. 2009; Nagajyoti et al. 2010). Heavy metals cause serious damage to living organisms by altering cellular signaling and by causing irreversible damage to biological systems (Rossato et al. 2011). Indeed, they may exhibit devastating effects on DNA structures by rising intracellular reactive oxygen species (ROS), by the cross-linking of proteins to DNA, or by changing nucleotides and sequences (Caicedo et al. 2008).

Some studies point out the significant role of vegetables in the transfer of metals from soil to the human consumer (Piotrowska and Kabata-Pendias 1997; Dugo et al. 2004; Reid et al. 2003). Many factors influence differences in the bioaccumulation of metals in vegetables for food (agricultural practices, transportation, harvesting processes, storage, and commercialization conditions) (Bovell-Benjamin 2007). Plants exposed to heavy metal toxicity have evolved mechanisms to protect themselves from the damages produced by metals, such as phytochelatins (small metal-binding peptides) and metallothioneins (metal-binding proteins). These levels increase in response to stressing agents, including metals and oxidative stress (Wawrzynski et al. 2006; Schat et al. 2002; Ouziad et al. 2005).

Great attention has been directed towards antioxidant compounds because of recent data describing the important role of antioxidants in human health as preventive and therapeutic agents. Many vegetables contain compounds (carotenoids, vitamins, polyphenols) with antioxidant activity, and an increased consumption of fruits and vegetables in the diet could reduce the risk of cancer and heart disease (Rissanen et al. 2003; Vainio and Weiderpass 2006). Among vegetables, the tomato is the most important both for its worldwide

consumption and for its plentiful use in health-related food components (Tommonaro et al. 2012; Tommonaro et al. 2013). Some studies have shown that the consumption of tomatoes and related products is associated with a lower risk of developing cancer of the digestive system and prostate (Giovanucci et al. 1995; Franceschi et al. 1994). As a consequence, tomatoes have acquired the status of a functional food.

This paper describes the evaluation of the nutritional quality of tomatoes grown in the toxic muddy soil in a contaminated area of Campania region. The level of contaminants as well as the content of heavy metals (Cd, Cr, Pb, Cu, Zn, Ba) is already reported in literature (Adamo et al. 2014). The presence of contaminants occurs because the sampling site is in an area with an uncontrolled outflow of industrial wastewater and agricultural practices characterized by the intensive use of pesticides and fertilizers.

The paper also reports on the presence of heavy metals in tomato fruit, with the aim of providing useful information for the promotion of food safety.

## Materials and methods

### Sampling

Sampling of tomato fruits (hybrid Rome type) was performed in September 2013 at maximum ripening stage in the San Mauro area of Nocera Inferiore. Here, the muddy soil along the Sarno River (Campania region, Italy) is one of the most contaminated areas in Campania. All tomatoes appeared similar in growth and ripeness. Fruits were a deep red color and without injuries.

### Extraction

All tomato fruit preparations, after the initial cleaning procedures, were performed with the separation of seeds and pulp. The pulp were homogenized in a blender, centrifuged at 9500 rpm for 20 min to obtain a hydrophilic fraction (supernatant) and pellet, while the seeds were crumbled in a mortar. Pellets and seeds powder were extracted with diethyl ether (*w*/*v* 1:2) under stirring at dark obtaining lipophilic extracts, which were then filtered and concentrated in a rotary evaporator in vacuum ($T<35$ ° C) and dried under $N_2$. In a separate experiment, samples were extracted with methanol for 2 h at 4 °C. The methanolic extracts were filtered and concentrated in a rotary evaporator in vacuum and dried under $N_2$.

### ABTS assay

Evaluation of antioxidant activity of lipophilic fraction of samples was performed according to the ABTS [2,2′-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)] method (Miller et al. 1996). The reaction mixture contained 56 mM ABTS and 24.5 mM $K_2S_2O_8$ in ethanol (dilution 1:100) in a total volume of 1 mL. The lipophilic fraction was added to the reaction mixture, and the decrease in absorbance at λ 734 nm was determined after 5 min at room temperature. The absorbance decrease was determined from the difference between the $Abs_{734}$ values before and after addition of sample. According to the ABTS method, the antioxidant activity of lipophilic fraction was carried out in triplicate on the diethyl ether extract of each sample, dissolved in dichloromethane analytical grade (20 mg/mL), and on its dilutions 1:2; 1:5; 1:10. The antioxidant activity was evaluated as percent of inhibition of radical cation $ABTS^{\cdot+}$. Results were reported as mean of three independent experiments.

### DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) test (Blois 1958) was carried out on methanolic extracts (20 mg/mL); 50 μL of these solutions were added to 0.7 mL of DPPH in MeOH (6 mg/50 mL; 0.1 mM final concentration) and adjusted to 2 mL final volume with MeOH. The absorbance at λ 517 nm was determined after 30 min at room temperature, and the percent of free radical inhibition was calculated. The antioxidant activity of samples was reported as percent of inhibition of $DPPH^{\cdot}$ free radical. Results were reported as mean of three separate experiments.

### DMPD assay

Antioxidant activity of hydrophilic fraction of all samples was evaluated by the DMPD (N,N-dimethyl-p-phenylenediamine dihydrochloride) method (Fogliano et al. 1999). Briefly, the reaction mixture contained 1 mM DMPD, 0.1 mM ferric chloride in 0.1 M acetate buffer (pH 5.25) in a total volume of 1 mL. Then, 5, 2.5, 1, and 0.5 μL of hydrophilic fraction were added to the reaction mixture and the absorbance was determined after 20 min at room temperature at λ 505 nm. The antioxidant activity of hydrophilic fraction was carried out in triplicate and expressed as percent of inhibition of radical cation $DMPD^{\cdot+}$.

### Polyphenols content

The total polyphenol content was measured using the Folin-Ciocalteau colorimetric method (Singleton and Rossi 1965); 50 μL of Folin-Ciocalteau's phenol reagent and a volume of samples ranging from 10 to 50 μL were added to 800 μL of deionized water, and accurately mixed. After 1 min, 100 μL of 20 % sodium carbonate solution was added and mixed. Deionized water was then added up to a volume of 1 mL. Total phenol content was spectrophotometrically estimated at

765 nm (DU-Beckman, USA), 2 h after incubation at room temperature, by using quercetin as standard curve. All determinations were carried out in triplicates.

Antiproliferative activity

PDAC, Hela, and A375 cells (American Tissue Culture Collection, ATCC), were grown in adhesion on Petri dishes and maintained at 37 ° C with DMEM supplemented with 10 % heat-inactivated FCS, 25 mM HEPES, 100 u/mL penicillin, and 100 μg/mL streptomycin. Cells ($3.5\times10^4$ cells/well) were plated on 96-well microtiter plates and allowed to adhere at 37 ° C in a 5 % $CO_2$ atm for 2 h. Thereafter, the medium was replaced with 50 μL of fresh medium and 75 μL aliquot of serial dilution of each extract was added and then the cells incubated for 72 h. Mitochondrial respiration, an indicator of cells viability, was assessed by the mithocondrial-dependent reduction of [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2H-tetrazolium bromide] (MTT) to formazan, and cells' viability was assessed accordingly to the method of Mosmann as previously reported (Tommonaro et al. 2014). Briefly, 5 μL of MTT (5 mg/mL) were added and the cells were incubated for 3 h. Thereafter, the cells were lysed and the dark blue crystals solubilized with 100 μL of a solution containing 50 % (*v*:*v*) N,N-dimethylformamide, 20 % (*w*:*v*) SDS with an adjusted pH of 4.5. The optical density (OD) of each well was measured with microplate spectrophotometer (Titertek, Multiskan MCC/340) equipped with a 620-nm filter. The viability of each cell line in response to treatment with tested extract was calculated as: percent dead cells=100−(OD treated/OD control)×100.

Atomic absorption spectroscopy

All the metals, Cu, Zn, Pb, Cr, Ba, Cd, were determined by AAS using a Perkin Elmer AAnalyst 100 equipped with a Perkin Elmer HGA 850 graphite furnace and a Perkin Elmer AS 800 autosampler. Every standards and sample were diluted in absolute ethanol purchased from Sigma-Aldrich. The analytical calibrators were purchased from Carlo Erba. The range of standard curve was built from $5\times10^{-3}$ to 4 mg/L. Laboratory glassware for analytical purposes was cleaned with $HNO_3$ 2 % and rinsed with abundant deionized water before use. The occurrence of contamination, possibly arising from the autosampler, is avoided with an accurate wash with acid water at 3 %.

## Results

The sampling of tomatoes was performed in September, at time of maximum ripening and production. Nutritional analysis of tomato samples was carried out with the aim to verify the presence of heavy metals in fruits and to investigate the effect of these contaminants on the known antioxidant capacity of tomatoes.

The weight of the fresh tomatoes and the yield of lipophilic and methanolic extracts are summarized in Table 1

We evaluated the antioxidant activity of lipophilic and methanolic fractions of tomato pulp and seeds by means of ABTS and DPPH assays, respectively. Moreover, we estimated the antioxidant capacity of hydrophilic fraction of tomato pulp by using DMPD assay (Fig. 1). As expected, tomato pulp exhibited a great antioxidant capacity, both for the lipophilic and methanolic fractions, in comparison with seed extracts. In particular, at maximum tested amount of diethyl ether extract (100 μg), we observed an inhibition of radical cation $ABTS^{\cdot+}$ of 100 %, and a reduction of activity decreasing with the tested amounts up to 35 % corresponding to 10 μg. As regards the methanolic extracts, tomato pulp showed a maximum free radical scavenging capacity (45 % of $DPPH^{\cdot}$ inhibition) at the amount of extract of 1 mg. Moreover, the polyphenolic content was estimated in methanolic extract by Folin-Ciocalteau method. Results showed that in 100 g of fresh fruits 5.77 meq of quercetin were present. Lipophilic and methanolic extracts of tomato seeds did not show a significant antioxidant activity evaluated by ABTS and DPPH assays at all tested amounts. Hydrophilic fraction of tomato pulp also exhibited a significant antioxidant capacity, estimated by means of DMPD assay. Indeed, 5 μL of aqueous fraction of tomato pulp was able to inhibit the cation radical $DMPD^{\cdot+}$ of 100 %. These results were surprising considering that tomatoes grew in muddy soils. The results of antioxidant assay showed that tomatoes keep a high antioxidant activity even in the presence of heavy metals (Table 2).

Methanolic and diethyl ether extracts of pulp and seeds tomato were evaluated for their possible toxicity on cells. Cytotoxicity assays have been performed on HeLa, PDAC, and A375 cell lines and the results, both for chloroformic and methanolic extracts, for the seeds and for tomato pulp are

**Table 1** Weight of the fresh tomatoes and the yield of lipophilic and methanolic extracts

| Tomatoes data yield | |
|---|---|
| Fresh tomatoes weight (g) | 224.7 |
| Total volume of mixed tomatoes (mL) | 230.0 |
| Volume hydrophilic fraction (mL) | 135.0 |
| Weight pellet (g) | 29.4 |
| Weight diethyl ether extract (g) | 33.7 |
| Weight methanolic extract (g) | 464.3 |

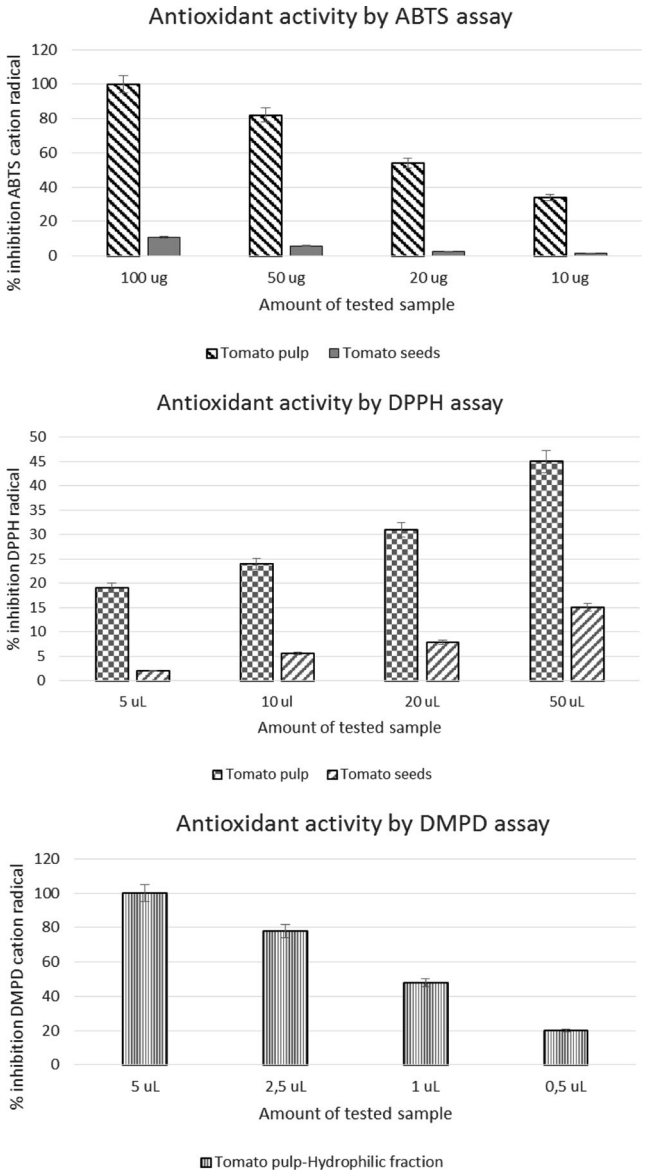


**Fig. 1** Antioxidant activity of different extracts of pulp and seed tomatoes

negative because they do not show a clear cytotoxicity. Indeed, all extracts showed an $IC_{50}$ value greater than 100 μg/mL. This result suggested the absence of heavy metals in the tested fractions.

The presence of some heavy metals (Cu, Zn, Pb, Cr, Cd, and Ba) in tomatoes has been evaluated using spectroscopy of atomic absorption with a graphite oven, and the performed tests confirmed the absence of heavy metals.

## Discussion

Luis et al. (2012) reported that samples of tomato from Morocco were analyzed for the determination of essential elements (Cu, Fe, Mn, and Zn) and heavy metals (Cd and Pb). All samples showed an interesting content of Fe, which was the most important trace element, followed by Zn, Mn, and Cu. As for heavy metals, mean levels of Pb were higher than Cd content, but the content of both metals did not exceeded the limit established for vegetable products by European legislation (DOUE L364/5-L364/24 2006). The presence of heavy metals in the soil could have an effect on the content and translocation of micro and macronutrients in tomatoes. Bertoli et al. (2012) reported the study on tomatoes grown under different condition of Cd concentration and the evaluation of essential element content in different parts of the tomato plant. Results showed that Cd reduced the content of K, Ca, Mn, and Zn in the aerial part; K in the fruit; and Mn in the roots. Moreover, Cd accumulated mainly in the roots.

Another study also investigated the minor elements such as Ca, Fe, K, Mg, and Na as well as trace elements such as Cd, Co, Cu, Mn, Ni, Pb, and Zn in 12 different species of vegetables, including tomatoes from Saudi Arabia. Tomatoes exhibited the highest level of Cu and Ni, though the element concentrations of these vegetables were within safety levels for human consumption (Mohamed et al. 2003).

Different organic matter (pig slurry, sewage sludge, manure, fish waste, etc.) is used as fertilizer in agricultural soils to improve plant growth and to obtain a successful harvest. Sewage sludge, in particular, deriving from municipal and industrial wastewater treatment, often is contaminated with Ni, a harmful element for food (Miner et al. 1997; Hyun et al. 1998). The effects of sludge application as organic fertilization in the presence of different levels of Ni on tomato fruit yield, quality, and nutrition were described in Palacios et al. (1999). Results showed that sewage sludge could be successfully used as horticultural fertilizer because it did not affect the quality and nutritional aspect of the tomato fruit. Only the highest addition of Ni (240 mg $Kg^{-1}$) had negative effects on tomato fruit yield and quality.

The results described in the present work agree with those reported above. The evaluation of the nutritional quality of

**Table 2** Cytotoxicity of lipophilic and methanolic extracts of pulp and seeds tomato

| | Cell line | | |
|---|---|---|---|
| Seed | HeLa | PDAC | A375 |
| Lipophilic extract | 139.74 μg/mL | 180.8 μg/mL | 181.19 μg/mL |
| Methanol extract | 550.2 μg/mL | 456.1 μg/mL | 656.06 μg/mL |
| Pulp | | | |
| Lipophilic extract | 576 μg/mL | 111.9 μg/mL | 179.76 μg/mL |
| Methanol extract | 243.07 μg/mL | 127.55 μg/mL | 406.253 μg/mL |

Results are expressed as $IC_{50}$ (μg/mL) values indicating the concentration of each extract that affords cell growth by 50 % as compared to control cells

tomatoes grown in toxic muddy soil and the checking of the presence of heavy metals in the tomato fruits has shown that tomatoes keep a high antioxidant activity even in the presence of heavy metals in the soil. It is known that plants have evolved mechanisms to protect themselves from metals toxicity by means of specific small peptides (phytochelatins) and proteins (metallothioneins) that are able to bind metals. Moreover, a promising field to exploit plant–endophyte partnerships is the remediation of contaminated soils. During phytoremediation of organic contaminants, plants can further benefit from endophytes possessing appropriate degradation pathways and metabolic capabilities which lead to more efficient contaminant degradation and reduction of phytotoxicity. For phytoremediation of toxic metals, endophytes possessing a metal-resistance/sequestration system can lower metal phytotoxicity and affect metal translocation to the aboveground plant parts. Furthermore, endophytes could offer promising ways to improve phytoremediation of mixed pollution (Weyens et al. 2009). This study may provide a better knowledge of the toxic metal content in food. Daily consumption of tomatoes improves the intake of essential microelements and bioactive compounds having antioxidant capacity. This study showed that the tomato consumption does not affect appreciably the intake of heavy metals and provides useful information for promoting food safety.

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