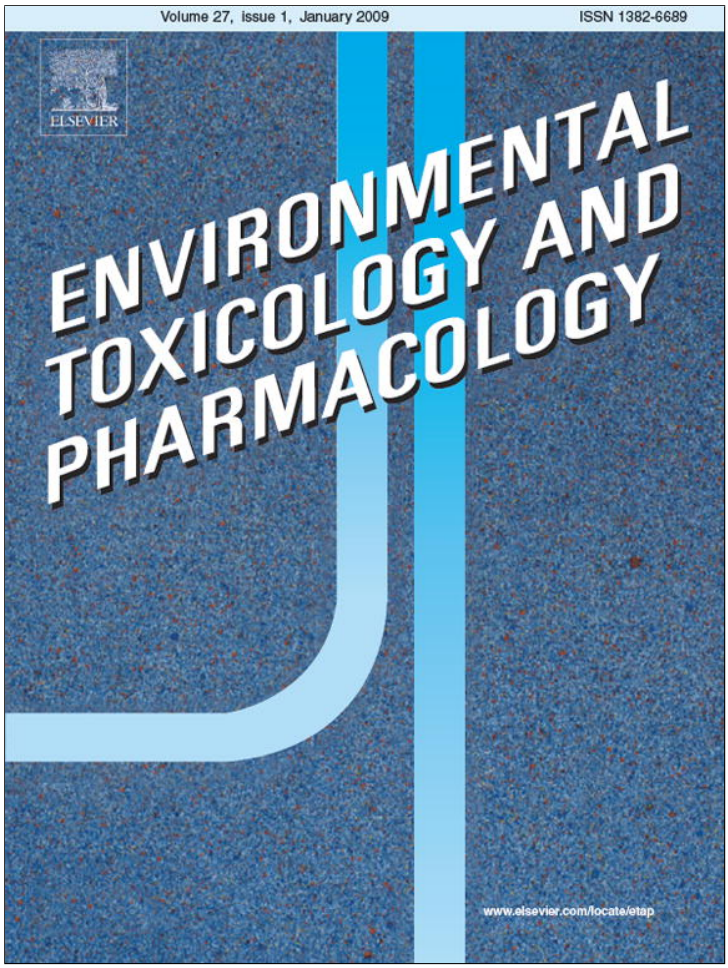

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Removal of radioisotopes in solution and bactericidal/bacteriostatic sterilising power in activated carbon and metal silver filters

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ABSTRACT

Activated carbon filters play an important role in water filtration and purification from contaminants of different origin. Their limit consists in bacterial proliferation, which may occur only during prolonged periods of non-use and in their ability to remove radioactive contaminants present in waste water from Industry or Nuclear Medicine departments.

In this work we tested a commercially available activated carbon filter for water purification enriched with silver plated parts incubating in static condition at room temperature different micro organisms (*Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Aspergillum niger*), up to 78 days.

The microbial growth was in general more inhibited in the presence of metal silver into the activated carbon in respect to filters with the activated carbon alone: >4 log inhibition of bacterial proliferation after 78 days of incubation the presence of silver vs. 2 log without silver. When the filters were incubated empty of carbon, the sterilizing power of silver was confirmed further.

The activated carbon filters proved also their ability in removing from water the principal radioisotopes used for residues liquid medical and research purposes (^{131}I, ^{99m}Tc, ^{201}Tl, ^{67}Ga).

These results contribute useful data for the use of the silver-enriched carbon filters in water filtration both for daily use at home, and professional use in a Nuclear Medicine laboratory.

1. Introduction

The important role that activated carbons play in the air flow hoods used in laboratories where radioisotopes are manipulated in order to hold volatile atoms, such as the ^{131}I, as well the capacity of these carbons to bond substances contained in a solution (biocides, pesticides, chlorides, and so on) is well documented (Abramns et al., 1986; Hager and Flentje, 1965; Hayes, 1966; Joyce and Sukenik, 1964; Li et al., 2006; Namane and Hellal, 2006; Sabio et al., 2006).

The introduction of drinking water disinfection with chlorine in the 20th, has greatly decreased the microbiological contamination. Chlorination produces a large numbers of halogenated compounds (e.g. chloroform, bromodichloromethane) declared as probable human carcinogens (Pedahzur et al., 1995).

The scientific and technological efforts in solving these problems are directed in several paths, for example UV irradiation, membranal processes, ozone, hydrogen peroxide, metal ions such Ag^+ or/and Cu^+ (Kim et al., 2002, 2004; Lin et al., 1996; Pedahzur et al., 1997).

The observation that pieces of copper, silver and other metals, when put into water are able to inhibit the proliferation of bacteria, algae and schizomycetes, traces back from age to age. Silver is a bacteriostatic/bactericidal agent (Chambers et al., 1962) which is used in point of use activated carbon based filters (Bell, 1991). There are many open questions about the possibility of bacteria proliferation in this filter, although it has been demonstrated that silver (Ag^+) exhibited a significant inactivation performance even at concentrations < 90 ppb, maximum contaminant level (MCL) that do not pose any health risk according to the USEPA, EEC, WHO.

The inhibitory action of silver can be attributed to its strong interaction with thiol groups present in respiratory enzymes of the bacterial cell. Additionally, silver has been shown to interact with structural proteins and preferentially bind to DNA bases to inhibit replication (Richard et al., 2002; Russel and Hugo, 1994).

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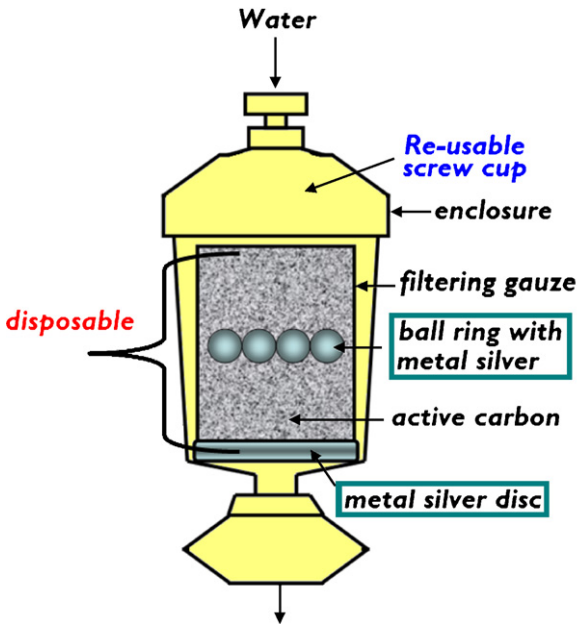

Fig. 1. Scheme of the activated carbon filter with silver.

The principal aim of this paper was to evaluate the performance and the characteristics of a commercially available activated carbon filter for water purification, bacteriostatic power of which was enriched with addition of metal silver. This aim was achieved in a first step by evaluation of the bactericidal–bacteriostatic power of such filters to self-sterilize from eventual bacterial proliferation along those periods (3 months) of inactivity when the filter is not being used, i.e. without continuously washing the filter; in a second step, by evaluating the filtration capacity of such filters in respect to solutions containing radioisotopes used for medical purposes, in particular, those used in Nuclear Medicine and in Pharmaceutical Industries research.

2. Materials and methods

2.1. Materials

2.1.1. Activated carbon filters and metal silver

A commercially available water filter (AQUASAN GM INTERNATIONAL Company, Italy) made of non biodegradable materials (biologically inert) containing activated carbons and one or more silver bound disks made with a new patented technology based on metal silver was used.

The filter is depicted in Fig. 1 and consists of: a re-usable enclosure in nontoxic polycarbonate (Lexan) and Kostil, food and water safe, and a disposable filtering gauze in polypropylene 5 mm thick, (biologically inert and nontoxic, food and water safe) with 50 μm pores, filled with 7 g of granular activated carbon Norit PK 0.25–1 (Norit Netherlans B.V., The Netherlands) with particles size from 0.25 to 1 mm, a specific surface area 800–1200 m^2/g, pores size 5–40 Å. Seated in the activated carbons there is a disk in ABS plastic of 50 mm in diameter silver plated and a ball ring in ABS plastic silver plated. The release of silver from these parts into water was demonstrated to be less than 0.03 mg/L (tested by atomic absorption). As the current EPA standard for silver in water is < 0.05 no problem for health of users exists. Other characteristics of the carbon filling are reported in Table 1. The purchaser recommendation is to replace the filter disposable part after flushing 1000 L.

The filters were provided into a blister pack and were opened under sterile laminar flow hoods just before the inoculation experiments took place.

2.1.2. Micro organisms

All micro organisms families used for this purpose derive from the original family "American Type Culture Collection" (ATCC): *Enterococcus faecalis* (ATCC N. 29212), *Escherichia coli* (ATCC N. 25922), *Pseudomonas aeruginosa* (ATCC N. 27853), *Salmonella enteritidis* (ATCC N. 13076), *Staphylococcus aureus* (ATCC N. 25923), *Aspergillum niger* (ATCC N. 16404). The lyophilized micro organisms were suspended in BMI medium (3 mL) and incubated for 4 h at 37 °C in thermostat. Then, 10 μL were diluted with 10 mL BMI and incubated overnight at 37 °C. Each bacteria suspension was then adjusted with sterile saline (sodium chloride 0.9%, p/v) to an initial rough concentration of 0.5 10^6 c.f.u./mL by turbidimetric method based on McFarland standards.

Table 1
Specifications, typical analyses and characteristics of the NORIT PK 0.25–1 activated carbon

Molasses number	Maximum 600
Particle size	1.0 mm maximum 10% (w/w) <0.25 mm, maximum 5
Apparent density	290 kg/m^3
Density backwashed and drained	255 kg/m^3
Iodine number	700 mg/g minimum
Ash content	10% (w/w)
Moisture (as packed)	3% (w/w)
Effective size	0.3 mm
Uniformity coefficient	1.5
Dechlorination halving value	2.5 cm
Ball-pan hardness	82
pH	Alkaline

2.1.3. Radioisotopes

Physiological solutions of the following salts: ^{99m}Tecnetium ($^{99m}TcO_4^-$), Sodium ^{131}Iodide (^{131}NaI), ^{201}Thallium Chloride (^{201}TlCl), ^{67}Gallium Citrate were used. In addition binding proteins solutions with ^{125}I and ^{57}Co (vitamin B12) were tested.

2.2. Methods

2.2.1. Antibacterial activity tests with micro organism in suspension

To monitor the antimicrobial efficacy of activated carbon filters with metal silver over time if the are used improperly, that is without cycling of water flow (on-off), the different micro organism test suspensions, as reported in Table 2, were inoculated (1 mL, 0.5 × 10^6 c.f.u./mL) into the disposable part of the filters (Fig. 1) which, then, were immediately soaked into 60 mL of sterile saline in a sterile bottle. All containers were tapered and kept at room temperature (22 °C/70 °F) in static position without any replacing of physiological solution. At different times (i.e. during the first 24 h and then in average every 15 days for a total period of 3 months, Figs. 2 and 3), the containers were stirred and 10 μl solution was taken from each culture and seeded, after proper dilution, in triplicate on Columbia agar plates with 5% ram blood. Plates were put in incubation for 48 h at 37 °C/100 °F, then counting of the specific micro organism seeded was performed using colony forming unit (c.f.u.) and the obtained values were related to 1 mL of solution. As a comparison, the same set of experiments was performed also with activated carbon filters prepared without the metal silver bound disk and the ball ring silver plated.

Table 2
Antibacterial micro organisms test

Sterlised containers	Carbon filter + Ag	Carbon filter – Ag
1–2	Saline only	Saline only
3–4	+ *Enterococcus f.*	+ *Enterococcus f.*
5–6	+ *Escherichia c.*	+ *Escherichia c.*
7–8	+ *Pseudomonas a.*	+ *Pseudomonas a.*
9–10	+ *Staphylococcus a.*	+ *Staphylococcus a.*
11–12	+ *Salmonella e.*	+ *Salmonella e.*
Sterlised containers	**Filter + Ag**	**Filter – Ag**
13–14	+ *Enterococcus f.*	+ *Enterococcus f.*
15–16	+ *Staphylococcus a.*	+ *Staphylococcus a.*
17–18	+ *Pseudomonas a.*	+ *Pseudomonas a.*
19–20	+ *Salmonella e.*	+ *Salmonella e.*
21–22	+ *Aspergillum n.*	+ *Aspergillum n.*
23–24	+ *Escherichia c.*	+ *Escherichia c.*

Containers 3–12: activated carbon filters with or without silver were filled with 1 mL (0.5 10^6 c.f.u./mL) of the indicated ATCC families and incubated for different times in 60 mL physiological solution in sterilized. Containers 1 and 2: activated carbon filters with or without silver were filled with 1 mL sterile saline and incubated for different times as containers 3–13.
Containers 13–24: empty filtering gauze without activated carbon but in the presence or not of silver was incubated following the same procedure of containers 3–12 in the presence of the indicated micro organisms.
For incubation conditions see Section 2.

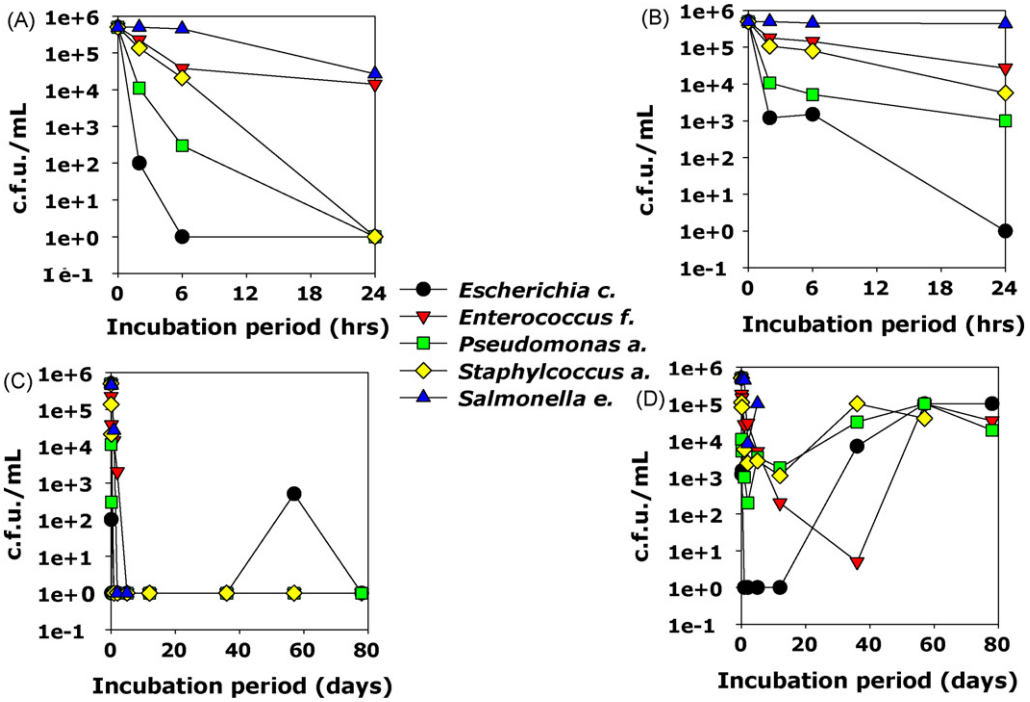

Fig. 2. Microbial growth in static saline incubated at room temperature for 24 h (A and B) and 78 days (C and D) in the presence of filters with activated carbon and metal silver (A and C), or activated carbon alone (B and D). The filters were filled with different micro organism test suspensions, immersed in 60 mL of sterile saline, tapered and incubated at room temperature in static position. At the indicated times, 10 μl were seeded in triplicate on agar plates, incubated for 48 h at 37 °C and the c.f.u. for each specific micro organism was counted and related to 1 mL of solution.

Moreover, to exclude any possible interference from activated carbon on the bacterial growth, a set of experiments was performed with the filtering gauze empty of the activated carbon, but in the presence or not of both the metal silver bound disk and the silver plated ball ring (Fig. 1, Table 2).

As a blank sample the growth of the microbial density in filters with or without metal silver was monitored also without any addition of the test micro organism.

2.2.2. Removal of radioisotopes in solution tests

The second set of experiments was intended to test the power of the new patented commercially available filters for water purification, to remove also a possible radioactive contamination. To this purpose, we used the commercially available device enriched with all the metal silver parts, although theoretically this activity should be ascribed only to activated carbon. Solutions of radioisotopes, carrier-free (^{131}I 0.0088 MBq; ^{99m}Tc 6.66 MBq; ^{201}Tl 3.4 MBq, ^{67}Ga 0.73 MBq) or bonded to

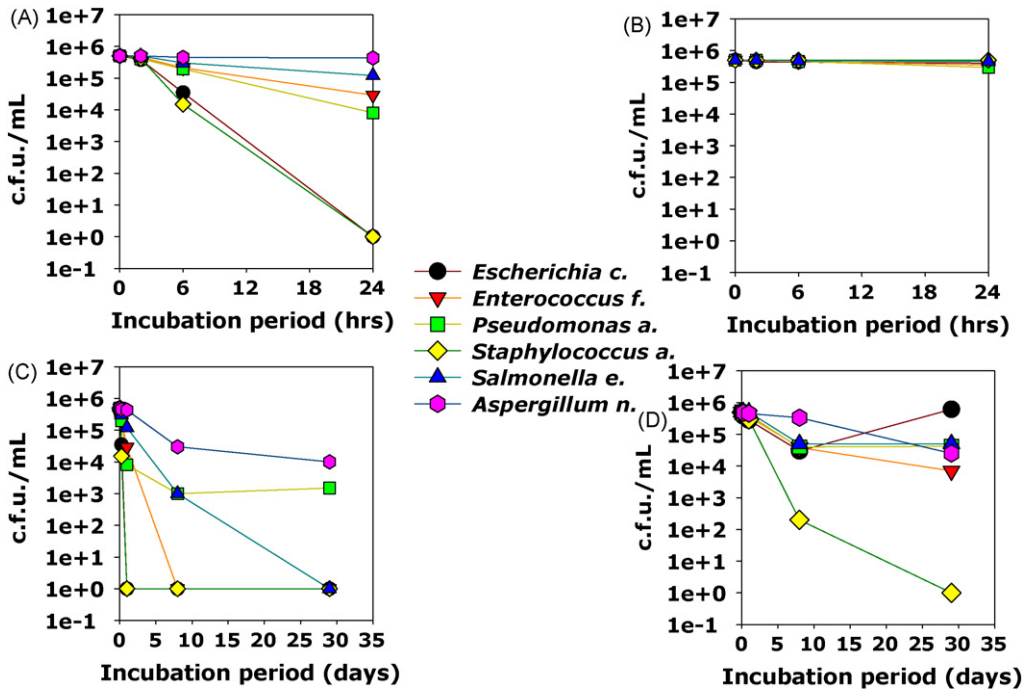

Fig. 3. Microbial growth in static physiological solution incubated at room temperature for 24 h (A and B) and 35 days (C and D) in the presence of filtering gauze with metal silver (A and C), or filtering gauze alone (B and D).

proteins (^{125}I 1.33 MBq; ^{57}Co vitamin B_{12} 0.0012 MBq), were introduced into the activated carbon filter with a syringe and then the filters were eluted with drinking water (6 L/min flow). After elution, the remaining radioactivity in the various parts of the filter (activated carbons, filtering gauze, and enclosure) was evaluated by removing the filtering gauze and separating the activated carbons. The radioactivity was evaluated by means of a NaI crystal probe, to reveal gamma emission such as: ^{131}I (main energetic peak 364 keV), ^{99m}Tc (main energetic peak 140 keV), ^{67}Ga (main energetic peak 300 keV) and ^{201}Tl (X, 81 keV). An automatic gamma counting system Kontron MR480 with programmed windows for ^{125}I (main energetic peak 35 keV) and ^{57}Co (main energetic peak 122 keV) was used. Each radioisotope was counted separately and the counting time was 1 min.

2.3. Statistical analysis

To compare the behaviour of the microbial density over time (0–3 months period) in the presence or not of metal silver, we expressed the microbial density at each time as % of the one at time 0 (T0), and pooled all the micro organisms family studied in the different experimental conditions. The significance of the differences ($p < 0.001$) was tested by the Mann–Whitney Rank Sum Test.

3. Results and discussion

3.1. Antibacterial activity tests with micro organism in suspension

The activated carbon filters with disk and ball-ring in metal silver demonstrated to provide a strong sterilisation power. In Fig. 2, the microbial growth into the static physiological solution in the presence of activated carbons filters with and without metal silver is reported for all the microbial families studied both in the first 24 h (Fig. 2A and B) and in the entire 78 days period (Fig. 2C and D). In the presence of metal silver the reduction of bacterial charge was >5 log (except for *Salmonella e.* and *Enterococcus f.*) after only 24 h of stasis at room temperature (natural condition of the filter during periods of non-use) and this result was almost constant for the entire period of the testing (3 months) (Fig. 2A and C). Substantially different results were observed with the filters without metal silver. In fact, after a physiological fall in the first days, which however never reached the 100%, there was a remarkable increase of the microbial charge after the 12th day with a peak of bacterial growth at 57th day (Fig. 2 B and D).

Despite the very high concentration of the test micro organisms suspensions inoculated at T0, an overall significantly lower ($p < 0.001$) bacterial growth was observed in the presence of metal silver in respect to filters without metal silver (according to directive no. 80/778/CEE dated 15 July 1980; the limit of the total content of total coli forms in water for human consumption is maximum 5 for 100 mL).

The confirmation of the above reported results came from containers 13–24 (Table 2), in which filters without activated carbon, but in the presence or not of both disk and ball-ring in metal silver, were incubated in the same conditions of containers 1–12. The activated carbons, in fact, could provide by itself a source of carbon which helps to develop the microbial charge.

The bacterial growth into the static medium in the presence of the filtering gauze with the ball ring and metal silver disk was significantly lower ($p < 0.01$) than the growth in the presence of the filtering gauze alone. In almost all cultures with metal silver, the microbial density was reduced >5 log after 35 days (except *Aspergillum n.* reduced 1 log and *Pseudomonas a.* reduced 1 and 2 log, respectively) (Fig. 3A and C), while in the static incubation devices with filtering gauze only without the presence of metal silver, the reduction never exceeded >1 log in the first 24 h and this result was confirmed a month later with no more than 2 log bacterial growth reduction (except for *Staphylococcus a.* for which a 6 log kill was anyway achieved) (Fig. 3B and D).

Furthermore, an important data was obtained from culture no. 1 (activated carbon filter with metal silver but without test micro organisms) (Table 2), where the sterilising power of metal silver lasted until the 78th day, and in culture no. 2 (activated carbons filter without metal silver and test micro organisms) where at the 36th day a bacterial proliferation of 7×10^3 c.f.u./mL was evidenced.

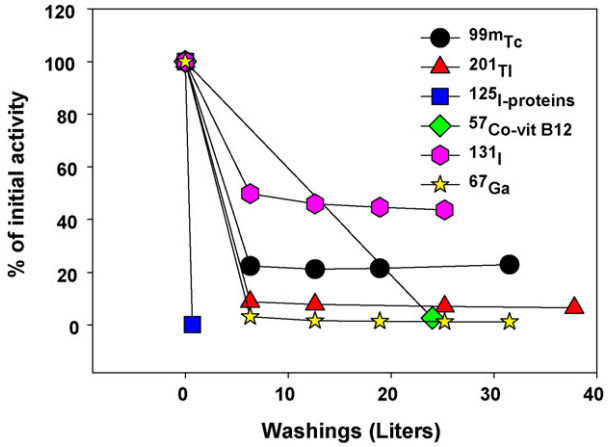

Fig. 4. Residual radioactivity in the metal silver activated carbon filter after elution with different washings volumes. Radioisotopes, carrier-free or bonded to proteins were introduced into the filter with a syringe and then the filter was eluted with drinking water (6 L/min flow). After the indicated elution volumes, the remaining radioactivity in the various parts of the filter (activated carbons, filtering gauze, and enclosure) was evaluated by removing the filtering gauze and separating the activated carbons. The radioactivity was evaluated by means of a NaI crystal probe, to reveal gamma emission. An automatic gamma counting system Kontron MR480 was used. Each radioisotope was counted separately for 1 min.

Table 3
Residual radioactivity in the filter components after elution with water

Filter parts	Radioisotopes (% of total activity loaded into the filter)			
	^{131}I	^{99m}Tc	^{67}Ga	^{201}Tl
Carbon	60.1	98.8	80.1	97.6
Filter cloth	34.4	1.1	19.0	2.2
Enclousure	5.4	0.1	0.9	0.2

3.2. Removal of radioisotopes in solution tests

In Fig. 4 the retention of radioisotopes onto the filters after elution with different washings volumes is reported. Activated carbon filters proved to be inefficacious for removal of ^{125}I bonded to proteins and for ^{57}Co bonded to vitamin B12. On the contrary, the filters displayed a good removal capacity for pure radioisotopes, those presents as ions in the solution. In particular, the ^{99m}Tc and ^{131}I were better bonded to the activated carbons than other radioisotopes (in the case of ^{99m}Tc, 23% of the initial activity remained bonded to the activated carbons still after 31.5 litres of elution). The residual radioactivity was found mainly bound to the activated carbons that showed the maximum bonding capacity with >97% of ^{99m}Tc and ^{201}Tl activity recovered in this part (Table 3). It is to underline that the quantity of activated carbons used in these tests is very small (7.0 g) and that larger quantities could be used if it is needed to bond a larger amount of radioisotopes.

4. Conclusions

Activated carbon filters have demonstrated an intrinsic capacity to proliferate bacteria, especially when, as shown by this work, they are not used for a period of time (data which supports Italian Legislation, D.M. no. 443 dated 21/12/1990 “Regulation containing technical dispositions for domestic potable water treatment appa-

ratus." In this law, article 51, it is written, "documented risks of bacterial proliferation exist in activated carbons filters")

On the other hand, activated carbons remove polluting substances (biocides, pesticides, chlorides, and so on) as well as radioisotopes in solution, as we demonstrated with this work.

Metal silver inserted into the filter and in contact with activated carbons has proved to have a strong sterilising power, even in consideration of the fact that at the initial test bacteria charges were very high. This killing action, likely to be ascribed to the silver ions released into the solution, is ensured for all the life period of the filters if they are used properly, i.e. replaced after 1000 L flushing.

From our data we conclude that activated carbon filters with metal silver can be used to purify water, in radioisotopes preparation industry, pharmaceutical industry, etc. since they impede the natural ability of bacteria to proliferate in activated carbon and they perform a sterilising process.

Conflict of interest

None.

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