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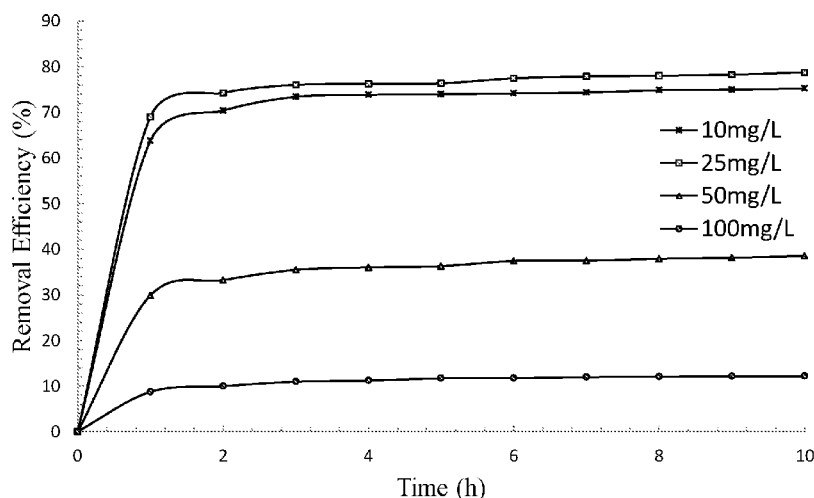
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FIGURE 1



(57) Abstract: According to the invention there is provided a biosorbent comprising at least one of watermelon rind, sugarcane bagasse and garden grass. Preferably, all three are present in synergistic quantities. Also provided for are methods of removing toxins from a material, remediating land and adsorbing metal/s from a material such as wastewater.

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BIOSORBENT FOR HEAVY METAL REMOVAL

Field of the Invention

The present invention relates to methods and processes for removing toxins
5 and in particular metals and heavy metals from various sources including water and
land. In certain embodiments this involves the use of biological materials to produce a
biosorbent for such toxins, metals and heavy metals, *e.g.*, copper, zinc and lead. The
biosorbent is preferably produced from waste agricultural materials. In some aspects
the present invention also provides methods pre-treatment and regeneration of a
10 biosorbent.

Background of the Invention

Any discussion of the prior art throughout the specification should in no way
be considered as an admission that such prior art is widely known or forms part of
15 common general knowledge in the field.

There is an ever-increasing awareness of toxins, heavy metals and other
various metals such as lead, copper and zinc in the environment. These metals cause
significant damage to the environment directly and also to humans and animals via
consumption of flora and fauna affected by such toxins.

20 Consumption of lead and its effects is well known. Copper is also a biological
poison and acute exposure in large doses can be harmful. The effects of other toxins
including metals, and in particular heavy metals, are well known in the art. It should
be understood, however, that the present invention is in no way limited to particular
toxins or heavy metals.

25 A multitude of processes and methods have been developed for the treatment
and disposal of metal-bearing wastewater so as to curtail heavy metal pollution issues
(*e.g.*, chemical precipitation, ion exchange, membrane separation, adsorption process,
and solvent extraction). However, these methods each have their respective
disadvantages, such as being relatively expensive, generating large amounts of sludge,
30 and involving either elaborate and costly equipment or high cost operation and energy
requirements. Therefore, there is a general need for the development of relatively cost-
effective and environmentally-friendly methods for remediating wastewater and the
like.

Extensive research has been carried out in an ongoing effort to develop a better
35 treatment for water and wastewater containing toxic heavy metals. A number of
innovative methods have been used to remove heavy metals, such as biosorption,
biosorption onto purified biopolymers, adsorptive filtration using coated sands, and

biosorption on magnetic iron oxides. Among these methods, biosorption has aroused interest.

Biosorption is the uptake of metals/substances by a biological means. “Biosorbents”, as applied herein, generally fall into several categories including
5 bacteria, fungi, algae, industrial wastes, agricultural waste and/or other polysaccharide materials. Agricultural waste or other biosorption processes which employ inexpensive dead biomass are particularly popular to sequester heavy metals from aqueous solutions, and are especially useful for the removal of trace amounts of heavy metals. The major advantages of biosorption include its low cost, high efficiency of heavy
10 metal removal from dilute solutions, cost-effective and simple regeneration of the biosorbent, the possibility of metal recovery, and the lack of nutrient requirements. Due to its excellent prospects, numerous materials have been studied for the development of cheaper and more effective biosorbents.

However, most biosorbent processes are metal-specific. The biosorbent is
15 chosen and prepared specifically to absorb a particular metal. Methods and processes are generally needed for a multi-metal system and for multiple uses.

Representative prior art includes CN 101601991, to Hunan University. This document discloses a biosorbent for removing lead ions in wastewater. The biosorbent is granular and uses calcium alginate and gelatin as carriers in which
20 grapefruit peel powder is embedded. The biosorbent is added into wastewater for proceeding adsorption treatment for at least 30 minutes at normal temperature in which pH value is 3.5-7.0, which can basically remove lead ions in the wastewater.

Similarly, US 2012/0024795 describes a biosorbent for removing cationic and/or anionic metals from aqueous solutions, and a process for the production of the
25 biosorbent. The biosorbent includes bacterial aggregates of *Bacillus sp.*, treated with polyethyleneimine and glutaraldehyde. Removal or recovery of metals from wastewater using the biosorbent is also described.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

30 Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”.

Although the invention will be described with reference to specific examples it
35 will be appreciated by those skilled in the art that the invention may be embodied in many other forms.

Summary of the Invention

According to a first aspect of the present invention there is provided a biosorbent comprising watermelon rind.

According to a second aspect of the present invention there is provided a
5 biosorbent comprising sugarcane bagasse.

According to a third aspect of the present invention there is provided a biosorbent comprising garden grass.

According to a fourth aspect of the present invention there is provided a biosorbent comprising at least two components selected from the group consisting of:
10 watermelon rind, sugarcane bagasse and garden grass. Preferably, the components are provided in quantities to synergistically enhance biosorptive activity. In an embodiment, the biosorbent comprises watermelon rind, sugarcane bagasse and garden grass. Preferably, these three constituents are provided in an approximate 1:1:1 ratio by mass.

15 In a preferred embodiment of the first through fourth aspects, the biosorbent is provided in a dry powdered form.

In another preferred embodiment of the first through fourth aspects, the biosorbent has a pH of about 6 to about 7; preferably about 6.5 to about 7; and most preferably about 6.8.

20 According to a fifth aspect of the present invention there is provided a method for removing toxins from a material, said method comprising contacting said material with a biosorbent as defined according to the first through fourth aspects of the present invention. In an embodiment, the material is a fluid. Alternately, the material is an aqueous solution. In an embodiment, the biosorbent is provided in a dosage of
25 between about 0.1 and 5.0 g/L.

According to a sixth aspect of the present invention there is provided a method of remediating land, said method comprising contacting said land with a biosorbent as defined according to any one of the first through fourth aspects of the present invention.

30 According to a seventh aspect of the present invention there is provided a method of adsorbing metal from a material, said method comprising one or more cycles of: a) contacting said material with a biosorbent as defined according to any one of the first through fourth aspects of the present invention; and desorbing said biosorbent. In an embodiment, the desorption step comprises contacting said
35 biosorbent with a suitable eluant for a predetermined period. In another embodiment, the method comprises up to 10 cycles. Alternatively, the method comprises up to 30-40 cycles.

In a preferred embodiment, the desorption step comprises contacting said biosorbent with one or more eluants selected from the group consisting of: distilled water, tap water, Milli-Q water, NaOH, HNO₃, HCl, H₂SO₄ and CH₃COOH. In an embodiment, prior to the contacting step, the biosorbent undergoes a pH pre-treatment to provide the biosorbent at a substantially neutral pH. Alternatively, prior to the contacting step, the biosorbent undergoes a pre-treatment comprising contacting the biosorbent with NaOH for a predetermined period.

In a particularly preferred embodiment of the fifth through seventh aspects of the invention, the biosorbent has a pH of between about 6 and 7. Most preferably, the biosorbent has a pH of about 6.8.

According to an eighth aspect of the present invention there is provided a method for adsorbing metals from a material, said method comprising contacting said material with a biosorbent as defined according to any one of the first through fourth aspects of the present invention.

In a preferred embodiment of the fifth through eighth aspects of the present invention, the metals comprise copper, zinc and lead, either alone or in combination.

In a preferred embodiment of the fifth through eighth aspects of the present invention, the material is water or wastewater.

According to a ninth aspect of the present invention there is provided a cosmetic formulation for topical application, said formulation comprising a biosorbent as defined according to any one of the first through fourth aspects of the present invention.

According to a tenth aspect of the present invention there is provided a method of removing heavy metals from a user's skin, said method comprising administering to said user an effective amount of a biosorbent as defined according to any one of the first through fourth aspects of the present invention, or of a cosmetic formulation as defined according to the ninth aspect of the present invention.

Having regard to the ninth and tenth aspects of the invention, any biosorbent or biosorbent-containing composition that contacts with a user's skin should ideally be substantially pH-neutral.

As mentioned above, biosorption is a process whereby metals or other substances can be adsorbed using a biological substrate. Agricultural waste, in particular, is a large potential source of biosorbent as it currently has no prominent utilisation.

Watermelon rind (*Citrullus lanatus*, family Cucurbitaceae) is a common agricultural by-product and natural and rich source of non-essential amino acid citrulline containing abundant carboxyl and amino functional groups which have a

remarkable capability of binding heavy metals from aqueous solutions. Studies have found that only half of a watermelon fruit is edible while the other half, consisting of about 30-35% rind and 15% peel goes to waste.

5 Bagasse is the fibrous matter that remains after sugarcane or sorghum stalks are crushed to extract their juice. There have been prior disclosures of sugarcane bagasse as an effective biosorbent, however, it has generally been used in isolation, *i.e.*, not in the synergistic combination proposed by the present invention.

“Garden grass” is self-explanatory; it is intended to encompass the clippings of any domestic lawn/parkland. Green grasses, rye grasses, *etc.*, are all envisaged.

10 It is understood that the adsorption of metal which is part of the biosorption process operates via the functional groups provided by the biosorbent materials. In conventional systems using only one or two biosorbents, only a small number of functional groups have provided, *e.g.*, 3 or 4 limiting both the quantity and type of metals which may be adsorbed. The presently-inventive biosorbent, however, appears
15 to provide a greater number of functional groups providing not only greater opportunity for adsorption of metals, but allowing a greater variety of metals which can be adsorbed.

The present invention also provides a synergistic combination of various biosorbent materials which are suitable for multi-metal systems. Although it is not
20 abundantly clear how this occurs, it appears that the combination of various biological materials in the inventive biosorbent has a synergistic effect in providing a greater number of functional groups than the cumulative number provided by each material. This synergistic effect, provides a “biosorptive capacity” which is several orders of magnitude greater than the individual components, or what would be expected by
25 simple aggregation of these components.

Such a surprising result was not expected by the Applicant. One may expect a cumulative effect at best or in some instances, a reduced biosorptive capacity due to competing biosorption dynamics of the various metals/toxins and biosorbents. Accordingly, the substantially improved biosorptive capacity of the combined
30 biosorbent mentioned above is a significant advance over conventional technologies.

In addition to the combined biosorbent, the Applicant has also elucidated biosorbents in the form of, simply, watermelon rind and garden grass. Again, the biosorption capabilities of these materials has to date, as far as the Applicant is aware, been unknown. As discussed below, watermelon rind is an inexpensive,
35 environmentally-friendly, stable and reusable biosorbent.

Similarly, garden grass has been found to be an excellent biosorbent for certain metals, *e.g.*, copper in water. The biosorption capacities of garden grass (as herein before defined) have to date been unreported.

5 **Brief Description of Drawings**

The present invention will now be described by way of example only with respect to the following drawings in which:

Figure 1 is a graph showing the effect of contact time of copper biosorption on a combined biosorbent in accordance with a preferred embodiment of the present invention.

Figure 2 is a graph showing the effect of pH on metal removal efficiency in a single metal solution using a biosorbent as indicated, *i.e.*, dosage: 0.5 g; particle size < 150 μm ; contact time: 10 h; initial metal concentration of Cu, Zn and Pb: 10 mg/L; 125 rpm; 20 $^{\circ}\text{C}$; NaOH: 0.1 mol/L.

Figure 3 is a graph showing the effect of pH on metal removal efficiency in a multi-metal solution using a biosorbent in accordance with a preferred embodiment of the present invention, *i.e.*, dosage: 0.5 g; particle size < 150 μm ; contact time: 10 h; initial metal concentration of Cu, Zn and Pb: 10 mg/L; 125 rpm; 20 $^{\circ}\text{C}$.

Figure 4 is an FTIR spectral analysis of a combined biosorbent in accordance with another embodiment of the present invention.

Figure 5 is an FTIR spectral analysis of a watermelon rind biosorbent in accordance with yet another embodiment of the present invention.

Figure 6 is an FTIR spectral analysis of sugar cane bagasse in accordance with yet another embodiment of the present invention.

Figure 7 is an FTIR spectral analysis of a garden grass biosorbent in accordance with yet another embodiment of the present invention.

Figure 8 is a graph showing the effect of pH on metal removal efficiency for copper, zinc and lead using watermelon rind as a biosorbent in a single metal solution.

Figure 9 is a graph showing the effect of pH on metal removal efficiency for copper, zinc and lead using watermelon rind as biosorbent in a multi-metal solution.

Figure 10A is a graph showing the effect of initial copper concentration and contact time using garden grass as a biosorbent.

Figure 10B is a graph showing the effect of pH on copper adsorption using garden grass as a biosorbent.

Figure 10C is a graph showing the effect of biosorbent dosage on copper removal, using garden grass as a biosorbent.

Figure 10D is a graph showing the effect of particle size of a biosorbent on the removal efficiency of copper using garden grass as a biosorbent.

Figure 11A shows the effect of desorption/regeneration of a garden grass biosorbent using various eluants; and

5 Figure 11B is a graph showing the effects of desorption/adsorption cycles of copper using garden grass as a biosorbent.

Best Mode for Performing the Invention

As mentioned above biosorption is the uptake of metals or other substances by
10 biological means. Generally, the biosorbent is obtained from agricultural materials such as wastes which are comprised of lignin and celluloses major constituents. They may also include other polar functional groups of lignin which include alcohols, aldehydes, ketones, carboxylates, phenols and ethers. These functional groups have the ability to some extent to bind heavy metals by donation of an electron pair from these
15 groups to form complexes with metal ion in solution.

Known biosorbents have included rubber wood dust, peanut shells, hazelnut husk, *Ceiba pentandra* hulls, banana peel, citrus peel, palm olive fruit shell, tree fern, Irish peat moss, cellulose pulp waste, wheat bran and micro algae. The present
20 Inventors, on the other hand, have found new and effective biosorbents which can be used either alone or in a synergistic combination.

Various experiments were conducted using watermelon rind as a biosorbent, garden grass as a biosorbent and a combined biosorbent formed from apparently synergistic quantities of watermelon rind, sugar cane bagasse and garden grass. It should be understood that the following experiments related to the uptake of certain
25 metals, in particular, copper, zinc and lead with the biosorbent. It will be understood by person skilled in the art, however, that the biosorbent may also be useful against other toxins and other metals, including heavy metals.

Further, it will be understood that the precise nature and contribution of each component in the combined biosorbent is not necessarily fully understood. Garden
30 grass, for example, in the combined biosorbent does appear to provide a synergistic effect in terms of the availability of functional groups. However, it is also believed that the garden grass provides a fibre substrate effect which could equally be provided by other fibre containing components, *e.g.*, flour. In this regard, experiments have been conducted using the flour, however, they were discarded on the basis of
35 difficulties in handling the flour and cost.

Various experiments were conducted to determine the nature and characteristics of biosorption process and in particular the combined biosorbent

containing watermelon rind and sugarcane bagasse and garden grass in synergistic quantities. The results of those tests will be discussed below.

“Combined” Biosorbent

5

Materials

Sugarcane bagasse and watermelon rind were collected from a local market while garden grass was collected from a recreational grass area. The collected biomass was washed with tap water and then rinsed with distilled water. Subsequently, the various components were dried, ground into powder and then mixed together in an approximate mass ratio of 1:1:1. Drying, as discussed below, was carried out in a laboratory-scale oven. The dried combined biosorbent was stocked in desiccator at room temperature (20 °C).

In regard to the garden grass, this was collected from a park in Campsie, New South Wales, Australia, after mowing. It was combined with three grasses, namely, Kikuyu grass (*Pennisetum clandestinum*) kangaroo grass (*Themeda australis*) and weeping grass (*Microlaena stipoides*). The grasses were not separated for the purposes of the experiment. It will be understood, however, that other garden grasses may also have suitability in the combined biosorbent.

All the chemicals used in this study were of analytical grade. Stock solutions of metal ions were prepared in MilliQ water. During the biosorption experiments, stock solutions were diluted to the specified concentration. The combined biosorbent was contacted with each solution at pH 6.78 (the approximate pH of tap water). The reaction mixture was agitated at 125 rpm on a shaker. Agitation contact time was kept for 10 h which was sufficient to reach equilibrium. The experiments were conducted at room temperature (20 °C).

Effect of contact time

Biosorption of metal with the novel combined biosorbent is a relatively quick process. Figure 1 is a graphical representation of removal efficiency versus time for copper adsorption. It can be seen from Figure 1 that at approximately two hours the biosorption process is close to its equilibrium since there is minimal increased removal after this point. Accordingly, it can be seen that an optimal contact time with the combined biosorbent may be four hours or less, preferably three hours, more preferably two hours or less. The most effective concentration appeared to be 25 mg/L. It should be recognised that this test was conducted with no pre-treatment

applied to the combined biosorbent. Later processes included a NaOH pre-treatment step.

Effect of pre-treatment

5 Further tests were then conducted to determine the effect of a pre-treatment step. Comparative adsorption tests were conducted both with and without NaOH pre-treatment.

To determine the effect of pre-treatment on metal uptake capacity of the combined biosorbent, an amount of dried biomass (0.5 g, for each procedure) was subjected to pre-treatment with 0.1 mol/L sodium hydroxide (NaOH). The raw combined biosorbent was allowed to contact 1 L of sodium hydroxide solutions for 10 h by stirring the mixture in a shaker at 125 rpm. The resultant material was washed with tap water after pre-treatment procedure until neutral pH (6.7-6.8) was measured, dried in an oven at 60 °C for 2 h, sieved again into a particle size ranging from 100 µm to 150 µm, and then stored in a desiccator until use.

The results of metal removal efficiency of such a combined biosorbent in a multi-metal solution both without pre-treatment (Table 1) and with pre-treatment (Table 2) are shown below.

20 *Table 1*

Metal removal efficiency of combined biosorbent in multi-metal solution (Dosage: 0.5 g; particle size < 150 µm; contact time: 10 h; pH: 6.78; 125 rpm; 20 °C)

Initial Metal Concentration of Cu, Zn and Pb (mg/L)	10	25	50	100
Cu removal efficiency (%)	75.28	78.75	38.51	12.23
Zn removal efficiency (%)	30.58	12.19	4.04	1.06
Pb removal efficiency (%)	95.25	96.47	73.22	64.59

25 *Table 2*

Metal removal efficiency of combined biosorbent in multi-metal solution (Dosage: 0.5 g; particle size < 150 µm; contact time: 10 h; pH: 6.78; 125 rpm; 20 °C)

Initial Metal Concentration of Cu, Zn and Pb (mg/L)	10	20	50	100
Cu removal efficiency (%)	99.99	19.99	48.99	98.42
Zn removal efficiency (%)	99.99	18.87	22.21	25.07
Pb removal efficiency (%)	99.99	19.99	49.99	98.91

In can be seen that in the case of the combined biosorbent, pre-treatment significantly improves the removal efficiency of metals. Although not entirely understood, it is believed that the NaOH pre-treatment may increase the surface area of the combined biosorbent and thereby activate more suitable binding sites. Further, it is possible that more functional groups (*i.e.*, OH) are added to the surface of the combined biosorbent. Accordingly, more micro-precipitation/adsorption will occur on the binding sites so as to remove the target ion, *e.g.*, metal, heavy metal, *etc.* Also, the negative charged surface can result in an attraction between the combined biosorbent and the target ion. Also strong physical adsorption means more metal ions can be removed.

Effect of pH

Further testing was then conducted to determine the effect of pH on metal removal efficiency.

Reference is made to Table 3 and Figure 2 in which the metal removal efficiency of the combined biosorbent in a single metal solution was conducted with varying pH.

Table 3

Effect of pH on metal removal efficiency in single-metal solution using combined biosorbent (Dosage: 0.5 g; particle size < 150 μ m; contact time: 10 h; initial metal concentration of Cu, Zn and Pb: 10 mg/L; 125 rpm; 20 °C)

pH		2	3	4	5	6	6.8
Metal Removal Efficiency (%)	Cu	5.79	17.89	27.84	48.36	94.75	99.93
	Zn	2.38	8.79	12.43	15.37	27.48	93.32
	Pb	89.99	93.48	98.97	99.97	99.67	99.97

25

It can be seen from Table 3 and Figure 2 that in regards to Pb, this metal is less affected by pH than is Cu or Zn. The effect of pH on metal removal efficiency of Zn is quite marked. Removal of Zn at very low pH, *e.g.*, 2-5, is around 15% or below. Metal removal efficiency increased markedly, however, as pH approaches neutral. At a pH of 6.8 metal removal efficiency of Zn was above 90%.

30

Cu, on the other hand, is also affected but less dramatically than Zn. For instance, at a pH of 5, metal removal efficiency for Cu is around 50% rising to above 90% at pH 6 and above.

Table 6

Effect of particle size (Dosage: 0.5 g; metal concentration of Cu, Zn and Pb: 10 mg/L; particle size < 150 μm ; contact time: 10 h; pH: 6.78; 125 rpm; 20 °C)

Size	Fine powder	Semi-Fine powder	Middle-Size powder	Semi-Coarse powder	Coarse powder
Diameter (μm)	<150	150-300	300-420	420-600	600-900
Maximum Removal Efficiency (%)	99.9	99.9	99.9	99.9	99.9

5

Further testing was conducted with the novel combined biosorbent to determine the effect of initial metal concentration on uptake in multi-metal solutions. The results are shown in Table 7. In this regard, it can be seen that in each instance after a 10 hour contact time nearly all Cu and Pb was adsorbed with the initial metal concentration was at 100 mg or less. Zn, on the other hand, seems to reach a maximum uptake of around 25 mg irrespective of the initial metal concentration. At concentrations above 100 mg metal removal efficiency of Cu reduces as does lead but to a lesser extent. Accordingly, it can be seen that again the novel combined biosorbent is an extremely robust and effective biosorbent over a wide range of initial metal concentrations.

15

Table 7

Effect of initial metal concentration on metal uptake in multi-metal solution (Dosage: 0.5 g; particle size < 150 μm ; contact time: 10 h; 125 rpm; 20 °C)

20

Initial metal concentration (mg/L)	Cu uptake (mg)	Zn uptake (mg)	Pb uptake (mg)
5	4.99	4.99	4.99
10	9.99	9.99	9.99
20	19.99	18.87	19.99
35	34.89	23.27	34.94
50	48.99	24.21	49.99
75	74.01	25.02	73.87
100	98.42	25.07	98.91
150	110.58	25.09	147.56
200	114.28	25.06	169.87
300	116.37	25.01	173.23

The Applicant also wishes to determine the effect of biosorbent dosage on metal removal efficiency, *i.e.*, whether increased biosorbent necessarily leads to an increase in metal adsorption. Results in this regard are shown in Table 8.

It can be seen that even at extremely low dosages of biosorbent metal removal efficiency is quite high. However, it can be seen that biosorbent dosages above 0.5 g work best for Cu. The novel combined biosorbent is extremely efficient at removing Cu irrespective of the biosorbent dosage. Even at the biosorbent dosage of 0.1 g Pb removal efficiency is over 97%. Zn metal removal appears to increase in proportion to biosorbent dosage.

The desorption characteristics and regeneration ability of the combined biosorbent was also investigated. After each desorption the biosorbent was contacted with a suitable eluant. Several eluants were used to test desorption characteristics, including tap water, Milli-Q water, distilled water, NaOH, HNO₃, HCl, H₂SO₄, or CH₃COOH.

15

Table 8

Effect of biosorbent dosage on metal removal efficiency in multi-metal solution (initial metal concentration of Cu, Zn and Pb: 100 mg/L; particle size < 150 μm; contact time: 10 h; 125 rpm; 20 °C)

20

Biosorbent dosage (g)	Metal removal efficiency (%)		
	Cu	Zn	Pb
0.1	68.54	2.92	97.53
0.2	70.90	5.65	97.88
0.5	96.49	24.03	98.30
1	97.01	29.75	98.94
2	97.83	33.06	99.07
5	98.47	59.28	99.27

Tables 9, 10 and 11 all relate to metal removal efficiency following ten rounds of adsorption and resorption with initial metal concentrations of copper, zinc and lead of 25, 50 and 100 mg/L, respectively. All other conditions were the same throughout these three experiments.

25

It can be seen that there is a substantially negligible reduction in the metal removal efficiency of the combined biosorbent even after 10 resorption cycles. Accordingly it is estimated that the novel combined biosorbent can be reused at least 30 to 40 times. This is a remarkable result and is clearly a significant contribution over the prior art.

30

Table 9

Metal removal efficiency in ten rounds re-sorption studies in multi-metal solution

(Dosage: 0.5 g; initial metal concentration of Cu, Zn and Pb: 25 mg/L; particle size <

5 *150 μm; contact time: 10 h; pH: 6.78; 125 rpm; 20 °C)*

Round	Cu removal efficiency	Zn removal efficiency	Pb removal efficiency
First round sorption	98.52%	88.24%	99.47%
Second round re-sorption	98.47%	88.67%	99.54%
Third round re-sorption	98.64%	87.89%	99.51%
Fourth round re-sorption	98.48%	88.24%	99.33%
Fifth round re-sorption	98.42%	87.28%	99.47%
Sixth round re-sorption	97.89%	88.04%	99.27%
Seventh round re-sorption	98.01%	88.14%	99.01%
Eighth round re-sorption	97.88%	87.99%	99.12%
Ninth round re-sorption	97.56%	87.47%	99.04%
Tenth round re-sorption	97.48%	87.52%	98.99%

Table 10

Metal removal efficiency in ten rounds re-sorption studies in multi-metal solution

10 *(Dosage: 0.5 g; initial metal concentration of Cu, Zn and Pb: 50 mg/L; particle size <*

150 μm; contact time: 10 h; pH: 6.78; 125 rpm; 20 °C)

Round	Cu removal efficiency	Zn removal efficiency	Pb removal efficiency
First round sorption	98.99%	62.12%	98.04%
Second round sorption	98.58%	60.78%	98.21%
Third round sorption	98.77%	59.86%	97.86%
Fourth round sorption	98.32%	58.43%	98.11%
Fifth round sorption	98.22%	57.99%	98.24%
Sixth round sorption	97.99%	58.02%	98.44%
Seventh round sorption	98.02%	58.47%	98.27%
Eighth round sorption	97.88%	57.89%	98.37%
Ninth round sorption	98.15%	57.28%	98.07%
Tenth round sorption	98.07%	57.01%	98.11%

Table 11

Metal removal efficiency in ten rounds re-sorption studies in multi-metal solution (Dosage: 0.5 g; initial metal concentration of Cu, Zn and Pb: 100 mg/L; particle size < 150 μm ; contact time: 10 h; pH: 6.78; 125 rpm; 20 $^{\circ}\text{C}$)

5

Round	Cu removal efficiency	Zn removal efficiency	Pb removal efficiency
First round sorption	96.49%	24.03%	98.30%
Second round sorption	95.59%	24.31%	98.41%
Third round sorption	96.01%	23.28%	98.31%
Fourth round sorption	96.13%	21.21%	98.24%
Fifth round sorption	96.27%	22.04%	98.56%
Sixth round sorption	96.33%	22.14%	97.89%
Seventh round sorption	96.07%	22.08%	98.01%
Eighth round sorption	95.89%	21.88%	97.48%
Ninth round sorption	95.75%	21.47%	97.01%
Tenth round sorption	95.81%	21.01%	97.11%

Table 12

Kinetic study of metal uptake (Dosage: 0.5 g; initial metal concentration of Cu, Zn and Pb: 10 mg/L; particle size < 150 μm ; contact time: 10 h; pH: 6.78; 125 rpm; 20 $^{\circ}\text{C}$)

10

Time (min)	Cu uptake (mg)	Zn uptake (mg)	Pb uptake (mg)
1	9.74	9.48	8.54
2	9.79	9.59	9.31
3	9.81	9.64	9.88
4	9.83	9.65	9.97
5	9.86	9.60	9.89
10	9.87	9.68	9.94
15	9.89	9.66	9.99
20	9.94	9.67	9.96
25	9.91	9.62	9.92
30	9.9	9.63	9.89
45	9.95	9.61	9.97
60	9.94	9.69	9.93

It will also be of interest to one skilled in the art that the biosorption process with the combined biosorbent was found to be somewhat fast. Most of the heavy

metals in solution can be adsorbed within the first five minutes of contact, offering great flexibility for practical applications. In this regard we refer to Table 12, which clearly shows that in many cases the vast proportion of metal uptake occurs within the first few minutes and in some cases within the first minute.

5 Table 13 shows the biosorptive capacity (in mg/g) of each of the three biosorbent constituents (banana peel, sugarcane bagasse and watermelon rind). The skilled addressee will note that the results for copper and zinc display marked synergy. The Table provides comparative data of the biosorptive capacity of the combined biosorbent compared with other biosorbents. It can be seen that the novel combined
10 biosorbent has a biosorptive capacity against conventional biosorbents such as banana peel and sugarcane bagasse, several orders of magnitude higher for all metal types shown.

Table 13

15 *Demonstration of the synergistic biosorptive effect for copper and zinc using the combined (1:1:1 by mass) biosorbent*

Biosorbent	Metal type	Biosorptive capacity (mg/g)
Banana peel	Cu	10.10
	Zn	6.17
	Pb	142.85
Sugarcane bagasse	Cu	10.64
	Zn	4.05
	Pb	122.75
Watermelon rind	Cu	6.28
	Zn	6.85
	Pb	92.88
Combined biosorbent (1:1:1)	Cu	140.86
	Zn	99.01
	Pb	270.27

20 Although not completely understood and not wishing to be bound by any particular theory, it is hypothesised that the adsorption of metal on the inventive combined biosorbent is as a result of functional groups available. With particular reference to Figures 4-7, the addressee will note the Fourier Transform Infrared Spectroscopy (FTIR) analyses of the novel combined biosorbent as well as its individual components, namely, watermelon rind, sugarcane bagasse and garden grass.

These analyses provide an interesting and unexpected result, namely, that the functional groups provided by the combined biosorbent are different in both number and characteristic than in the individual combined biosorbents. As exemplified in Table 14, the combined biosorbent has a greater number of functional groups as well as different functional groups than the individual components.

Table 14

Comparison of functional groups between CBS and single biosorbent

Biosorbent	Functional groups
“Combined”	carboxyl, alkyl, amine, phosphine, sulfur, hydroxyl
Watermelon rind	carboxyl, hydroxyl, alkyl, phosphine
Sugarcane bagasse	carboxyl, alkyl, phosphine, amine, hydroxyl
Garden grass	carboxyl, alkyl, phosphine, hydroxyl

10

It can be seen that the functional groups provided by the combined biosorbent do not result from the cumulative functional groups of the individual components. Rather, there appears to be a synergistic effect in the combining of these individual biosorbents to provide a biosorbent with a different functional group profile as well as a substantially enhanced biosorptive capacity over the individual biosorbents mentioned above. This appears to be a significant contributing factor to the functional capabilities of the inventive combined biosorbent having watermelon rind, sugar cane bagasse and garden grass in apparently synergistic quantities.

The Applicant has also found that watermelon rind and garden grass as individual biosorbents can provide advantages over the prior art. These biosorbents are discussed below.

Watermelon Rind Biosorbent

25 Materials

Watermelon rind was collected from a local market. The collected watermelon rind was washed with tap water and then rinsed with distilled water. Subsequently, watermelon rind was cut into small pieces, dried, and grounded into powder before its use in biosorption experiments. The drying experiments were carried out in a laboratory scale oven. Dried watermelon rind was stocked in a desiccator at room temperature (20 ± 1 °C).

30

All the chemicals used in this study were of analytical grade. Stock solutions of metal ions were prepared in Milli-Q water. During the biosorption experiments, stock solutions were diluted to the specified concentration. Watermelon rind was contacted with each solution at pH 6.48 ± 0.1 (the approximate pH of tap water). The reaction mixture was agitated at 125 rpm on a shaker. Agitation contact time was kept for 10 h, which was sufficient to reach equilibrium. All the samples from the experiments were filtered through a $0.45 \mu\text{m}$ nylon membrane filter and the filtrate was kept for analysis. Biosorption experiments were conducted in triplicate and average values were used for discussion. The whole experiment was conducted at ambient room temperature (20 ± 1 °C).

Effect of pH

Among various influencing factors, the solution pH can play a critical role in biosorption. It can affect the solution chemistry of metals and the activity of the functional groups of the biosorbents and can even completely inactivate the activity of binding sites. For metals ions, the speciation and biosorption availability can also be strongly affected by solution pH. Under the condition of higher solution pH, the solubility of metal complexes decreases, which may subsequently, lead to precipitation, complicating the biosorption process. This explains why experiments were conducted within the approximate acid-to-neutral pH range.

The effect of pH on biosorption efficiency was studied in the range from pH 2 to 6.8. The results for the biosorbent produced from watermelon rind are shown in Figure 8. This Figure shows the effect of solution pH on removal efficiency using watermelon rind as a novel biosorbent in a single metal solution. The dosage was 0.5 g with an initial metal concentration of 10 mg/L. Particle size was less than $150 \mu\text{m}$ with a contact time of 10 hours, agitation of 125 rpm at 20 °C. As shown in Figure 8, the maximum uptake of Cu took place at around pH 5. The uptake of Cu increased with increasing solution pH from 2.0 to 5.0 and then showed a slightly decreasing trend when pH was higher than optimal pH. This seems to indicate that for this biosorbent, ion exchange can be dominant in the biosorption of metal ions on watermelon rind. Therefore, at lower pH values, the biosorption capacities were reduced because of the competition between the large quantities of proton and metal ions for surface active sites. As the pH increased, the competition became less fierce and removal efficiency then increased. However, when pH increased over 7 the biosorption capacity became difficult to estimate.

For the biosorption of Zn and Pb, similar trends were found and the maximum uptake of Zn and Pb both occurred at around pH 6.8. It was also found that the uptake

of Cu and Zn could be more easily affected by pH alteration than the uptake of Pb, as the removal efficiency of Pb showed no clear difference when the pH changed from 5 to 6.8

5 Effect of coexistence of ions/multi-metal solutions

The effect of coexistence of ions for competitive biosorption was also tested. The results can be shown in Figure 9. This graph shows the effect of solution pH on removal efficiency using watermelon rind as a biosorbent in a multi metal solution. Again the dosage was 0.5 g with an initial metal concentration of 10 mg/L. Particle size was <150 µm with a contact time of 10 hours, agitation of 125 rpm at 20 °C.

Competitive biosorption is a common phenomenon with various biosorbents for metal uptake. The distinct characteristics of binding sites and certain functional groups on biosorbent surfaces result in high selectivity towards metal biosorption. Figure 9 provides the results of competitive biosorption for a multi-metal solution using watermelon rind as a biosorbent.

It was found that watermelon rind selectively absorbed Pb during the entire biosorption process with a reduced amount of Cu adsorbed. The uptake of Zn appears substantially reduced with the maximum removal efficiency of Zn found to be less than 10%.

Table 15 shows a comparison of removal efficiency of the three metals in a single-metal solution and a multi-metal solution using watermelon rind as a novel biosorbent. The pH for this comparison was 6.48 with an initial concentration of 10 mg/L. Other test criteria were as indicated.

25 *Table 15*

Comparison of removal efficiency of three metals in single-metal solution and multi-metals solution using watermelon rind as a novel biosorbent (pH, 6.48; initial metal concentration, 10 mg/L; particle size, <150 µm; contact time, 10 h; 125 rpm; 20 °C)

Metal type	Maximal removal efficiency (%)	Equilibrium time (h)
Cu in single-metal solution	58.4	1
Cu in multi-metals solution	55.6	1-2
Zn in single-metal solution	63.2	1
Zn in multi-metals solution	9.87	1-2
Pb in single-metal solution	99.9	1
Pb in multi-metals solution	93.1	1

The maximum capacity of Pb adsorbed was about ten times higher than that of Zn adsorbed, indicating that watermelon rind favours Pb biosorption. These results clearly show that watermelon rind is an excellent biosorbent for the separation of Pb from wastewater. It was also found that the co-existence of these metals reduced the maximum biosorptive capacities of watermelon rind for all three metals, with the uptake of Zn being inhibited to the greatest extent.

Effect of desorption

Desorption is an important part in the biosorption process for metal removal. As will be clear to persons skilled in the art, there is a need to desorb and recover the metal and thereby “regenerate” the biosorbent, at regular intervals. The efficiency of the regeneration of biosorbent after metal desorption also plays a vital role in the application of biosorption technology. Therefore, regeneration of biosorbents becomes significant. In large-scale applications, regeneration of the biosorbent has various benefits, such as keeping process costs down and recovering the metals extracted from the liquid phases. For this reason, environmentally-sensitive and inexpensive eluants become desirable to achieve non-destructive recovery so as to regenerate biosorbents for further reuse in multiple cycles.

To attain the above-mentioned objective, appropriate eluants are necessary, which are closely related with the type of biosorbent and the mechanism of biosorption. At the same time, an appropriate eluant should meet several requirements, such as yielding the metals in a concentrated form, no physical changes or damage to the biosorbent, and restoring the biosorbent close to the original condition for effective reuse with preferably undiminished metal uptake.

Being more cost-effective, environmentally friendly, and the efficacy of the biosorptive effect are also important criteria for choosing suitable eluants.

Four ordinary eluants were used for desorption of heavy metal ions (*e.g.*, distilled water, 0.1 mol/L NaOH, 0.5 mol/L HNO₃, and 0.5 mol/L HCl) from the watermelon rind biosorbent. In order to examine the reusability of this novel biosorbent, three rounds of biosorption-desorption cycle of Cu, Zn, and Pb in single-metal solution were conducted. Results are shown in Tables 16 and 17. Table 16 is for single metal solutions and Table 17 is for multi-metal solutions.

Based on the results shown in Table 16, it is clear that distilled water is non-effective while acidic eluants (*e.g.*, HNO₃, HCl) showed significant advantages in metal recovery. Almost 100% of metals ions were recovered. However, after acid desorption, the biosorptive capacity reduced significantly in the subsequent biosorption processes. For alkaline eluant (NaOH), it not only recovered most of the

adsorbed ions but also increased the biosorptive capacity. After desorption process in NaOH solution, the removal efficiency of three metals reached as high as 99% and remained constant for consecutive cycles. These results showed that watermelon rind could be repeatedly used in the biosorption process when alkaline eluants were used.

5 For comparison and better understanding of desorption characteristics, desorption and rebiosorption studies of Cu, Zn, and Pb in multi-metals solution were also carried out. As can be seen from Table 17, the results showed very similar trends as the results obtained in single metal solution. Remarkably, in comparison with the biosorption process, there was no significant competition between these three metals during the

10 desorption process. After desorption of NaOH, the removal of heavy metals became complete for all the three metals in the multi-metals solution even in the third biosorption-desorption.

Table 16

15 *Desorption and resorption studies of Cu, Zn, and Pb from watermelon rind in single-metal solutions using various eluants: distilled water, 0.1 mol/L NaOH, 0.5 mol/L HNO₃, and 0.5 mol/L HCl (pH, 6.48; initial metal concentration, 10 mg/L; particle size <150 μm; contact time 10 h; 125 rpm; 20 °C)*

Eluant	Metal type	First Cycle		Second Cycle		Third Cycle	
		Metal sorbed (mg)	Metal Desorbed (mg)	Metal resorbed (mg)	Metal Desorbed (mg)	Metal sorbed (mg)	Metal Desorbed (mg)
Distilled water	Cu	5.862	0.158	2.859	0.148	1.801	0.158
	Zn	6.111	0.165	4.892	0.168	3.481	0.169
	Pb	9.956	0.235	9.289	0.227	9.188	0.222
NaOH	Cu	5.862	2.319	9.935	5.927	9.965	5.837
	Zn	6.111	2.581	9.942	6.847	9.957	6.792
	Pb	9.956	7.148	9.958	7.211	9.999	7.182
HNO ₃	Cu	5.862	5.293	0.482	0.321	0.248	0.137
	Zn	6.111	5.892	0.473	0.304	0.222	0.108
	Pb	9.956	9.789	0.945	0.742	0.666	0.518
HCl	Cu	5.862	4.525	2.524	1.898	1.278	0.998
	Zn	6.111	5.292	3.072	2.565	0.892	0.563
	Pb	9.956	9.128	2.487	1.952	1.689	1.210

Table 17

Desorption and resorption studies of Cu, Zn, and Pb from watermelon rind in multi-metals solutions using various eluants: distilled water, 0.1 mol/L NaOH, 0.5 mol/L HNO₃, and 0.5 mol/L HCl (pH, 6.48; initial metal concentration, 10 mg/L; particle size <150 μm; contact time 10 h; 125 rpm; 20 °C)

5

Eluant	Metal type	First Cycle		Second Cycle		Third Cycle	
		Metal sorbed (mg)	Metal Desorbed (mg)	Metal resorbed (mg)	Metal Desorbed (mg)	Metal sorbed (mg)	Metal Desorbed (mg)
Distilled water	Cu	5.277	0.111	4.114	0.116	3.187	0.115
	Zn	0.987	0.541	0.618	0.329	0.428	0.239
	Pb	9.305	0.187	9.208	0.186	9.158	0.185
NaOH	Cu	5.277	0.411	9.944	6.608	9.743	6.548
	Zn	0.987	0.884	9.752	6.508	9.684	6.444
	Pb	9.305	7.064	9.921	7.492	9.948	7.462
HNO ₃	Cu	5.277	4.984	0.489	0.286	0.289	0.111
	Zn	0.987	0.956	0.217	0.111	0.117	0.052
	Pb	9.305	9.108	0.849	0.697	0.542	0.428
HCl	Cu	5.277	4.421	0.589	0.279	0.388	0.154
	Zn	0.987	0.649	0.456	0.234	0.218	0.145
	Pb	9.305	8.905	2.041	1.723	1.542	1.008

This efficacy of watermelon rind as a biosorbent is particularly surprising. It has been found after desorption NaOH, the adsorption of heavy metals improved and indeed became complete for all the three metals in the multi-metals solution even after a third biosorption–desorption cycle. This is, as far as the Applicant is aware, a novel and quite surprising effect. The explanation as to why this occurs is not completely understood, however it is believed that over the biosorption/desorption cycle the morphology of the biosorbent may have undergone a remarkable physical change. In this regard the textural parameters of the biosorbent throughout its cycle are summarised under Table 18.

It can be seen that the BET surface area of the biosorbents after the biosorption process are in fact larger than that of the raw biosorbent. Further, the surface area of the biosorbent after the desorption process is several orders of magnitude higher than after the sorption process. The Applicant believes that this goes at least some way to explaining why the biosorptive capacity of watermelon rind is increased significantly after the desorption process.

20

Table 18

Pore properties of various biosorbents (e.g., raw biosorbent, biosorbent after sorption process, and biosorbent after NaOH desorption process)

Property	Raw biosorbent	Biosorbent after sorption process	Biosorbent after NaOH desorption process
BET surface area (m ² /g)	5.97	15.55	82.87
Micro-pore area (m ² /g)	14.15	3.87	3.96
Micro-pore volume (cm ³ /g)	0.01	0.01	0.01
Mean micro-pore diameter (nm)	87.1	87.2	87.0

5

Green Grass Biosorbent

Materials

Grass is typically abandoned after mowing gardens, lawns, parks, *etc.* Several million tons of garden grass is mowed and dumped all over Australia each year. The Applicant has sought to prepare a bioadsorbent from this readily and nigh-infinitely available resource for metal removal from water, *e.g.*, copper, and desorption studies. The main objectives of this work were: (i) to characterise the physicochemical parameters such as specific surface area, surface morphology and structure, active groups *etc.*; (ii) to evaluate the effects of experimental conditions on copper removal such as pH, biosorbent dose, initial copper concentration, contact time, particle sizes and temperature; (iii) to determine the desorption capacity of copper and regeneration of biosorbent using various solvents; (vi) to determine the maximum adsorption and desorption capacities of garden grass based on several isotherm models; and (v) to discuss the adsorption and desorption kinetics of copper onto garden grass.

The garden grass was collected from Oswald Street Reserve, Campsie, New South Wales, Australia after mowing. It was combined of three types of grasses. The names of grasses were Kikuyu grass (*Pennisetum clandestinum*), Kangaroo grass (*Themeda australis*) and weeping grass (*Microlaena stipoides*) and in an attempt to make it user-friendly the grasses were not separated. Foreign matter was removed from the garden grass and washed with tap water and distilled water to remove dirt. The washed garden grass was kept in air to remove water from surface and then dried in oven at 105 °C for 24 h. The dried garden grass was grounded into powder and kept in air-tight bottle for later use.

A stock solution (1000 mg/L) of Cu^{2+} was prepared using copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in Milli-Q water. The working solution was prepared by diluting this stock solution with distilled water.

The effects of pH, garden grass doses, particle size, initial metal concentration (e.g., copper), contact time and temperature on metal adsorption were studied. The effect of initial copper concentration and contact time were conducted in 100 mL water with 10, 50 and 100 mg/L copper and 0.5 g garden grass for 7 hours at room temperature and non-adjusted pH. The pH experiment was done in 100 mL water with 0.5 g garden grass and the pH ranges were 2 to 8. Dosage effects were performed in 100 mL water with 0.05, 0.1, 0.5, 1 and 2 g of garden grass and with 1, 2.5, 5, 10 and 15 mg/L copper concentration. The effect of particle sizes were conducted in 100 mL water with 1-500 mg/L copper concentration and particle sizes were $>75 \mu\text{m}$, $75 \mu\text{m}$ and $150 \mu\text{m}$. The temperature effects experiment was conducted at 20, 30, 40, 50 and 70°C with 0.5 g garden grass.

Desorption of adsorbed copper from exhausted garden grass were studied with eight types of solvent including tap water, milli-Q water, distilled water, 0.1 N H_2SO_4 , 0.1 N HCl , 0.1 N HNO_3 , 0.1 N NaOH and 0.1 N CH_3COOH . Pre-adsorbed garden grass (0.5g) was taken in 100 mL of above mentioned medium and shaken at 120 rpm for 6 h. The eluted adsorbent was washed repeatedly with Milli-Q water to remove any residual desorbing solution and placed into metal containing water for the next adsorption cycle.

Effects of initial concentration

As exemplified in Figure 10A, the actual amount of copper adsorbed per unit mass of garden grass increased with the increase in copper concentration from 10 mg/L to 100 mg/L in the test water. As copper concentration in the test water was increased, unit adsorption of copper on garden grass increased from 14.06 to 137.12 mg/g. Maximum amount of copper was adsorbed within 400 min (6 hours) and equilibrium time for adsorption of copper onto garden grass was around 6 hours.

Effect of pH

As mentioned above, the pH of a solution affects surface charge of adsorbent and degree of ionisation and speciation of adsorbent. Normally, metal adsorption is dependent on pH condition of water. The effect of pH on the garden grass as a biosorbent for copper adsorption is exemplified in Figure 10B. The highest value of copper removal was achieved at a pH of around 6.0. At this value, the dominant species of copper was free Cu^{2+} ion which mainly involved in adsorption process.

Further adsorption test beyond this pH were hampered due to owing immediate precipitation of copper hydroxide.

Effect of biosorbent dosage

5 Effect of garden grass doses on copper adsorption are shown in Figure 10C. These were conducted at initial copper of 1, 2.5, 5, 10 and 15 mg/L, while the garden grass doses was varied from 0.5, 1, 2, 5, 10 and 20 g/L. The results indicate that the removal of copper rapidly increases with the increase in doses up to 5 g/L and thereafter remained unchanged. At equilibrium, removal increased from 50 to 84% for
10 an increase in dose from 0.5 to 5 g/L. The increase in copper removal is expected to be due to the increase in the available adsorption surfaces and sites. Maximum copper removal was found from 0.5 g/L garden grass dosage and 10 mg/L copper concentration.

15 *Table 19*

BET characteristics of garden grass

Parameter	Methods	Values
1. Surface area	BET surface area	21.28 m ² /g
	Langmuir surface area	-37.42 m ² /g
2. Pore area	DR method t-plot (statistical thickness – 3.50~7.00) Horvath-Kawazoe method	6.07 m ² /g
		0.12 m ² /g
1.11 m ² /g		
ii. Mesopore area	BJH adsorption	21.17 m ² /g
	BJH desorption	24.34 m ² /g
3. Pore volume	DR method t-plot (statistical thickness – 3.50~7.00) Horvath-Kawazoe method	0.00 cm ³ /g
		-0.01 cm ³ /g
0.00 cm ³ /g		
ii. Mesopore area	BJH adsorption	0.00 cm ³ /g
	BJH desorption	0.00 cm ³ /g
4. Pore size	DR method t-plot (statistical thickness – 3.50~7.00) Horvath-Kawazoe method	8.71 Å
		-1069.53 Å
14.98 Å		
ii. Mesopore size	BJH adsorption	40.18 Å
	BJH desorption	37.23 Å

Effect of particle size

Copper adsorption capacities at three particle sizes of garden grass are shown in Figure 10D. The monolayer adsorption capacity (q_m) of copper increased as the particle size of the garden grass decreased. Langmuir isotherm parameters q_m and K for each of the three particle sizes were calculated and are listed in Table 19. It is noteworthy that q_m for each particle size, increased from 6.064 to 11.173 mg/g with decreasing of particle size from 150 to $<75 \mu\text{m}$. This may be due to the larger specific surface area available for adsorption with smaller particles at a constant mass of garden grass during the process.

The specific surface areas of the garden grass biosorbent was calculated; the results are presented in Table 20. The maximum specific surface area of garden grass was $167.36 \text{ m}^2/\text{g}$ for $<75 \mu\text{m}$ particle size which is higher than BET surface area (*see*, Table 19).

Effect of desorption

Tests were also conducted to determine the desorption characteristics and regeneration ability of the garden grass. Eight types of eluants including 0.1 N H_2SO_4 , 0.1 N HCl, 0.1 N HNO_3 acids were used as eluant for copper desorption from garden grass. As shown in Figure 11A, adsorption of copper onto garden grass is easily regenerated by a small amount of 0.1 N H_2SO_4 . The results showed that the removal percentage of 95% of copper was realised with 0.1 N H_2SO_4 from Cu-loaded 0.5 g garden grass. For demonstrating the reusability of garden grass, the adsorption and desorption cycles were repeated five times. Although adsorption and desorption efficiency for the regenerated garden grass decreased gradually (*see*, Figure 11B), the regenerated garden grass could still be used five times with minor deviation of efficiency.

Further, the applicability of a bioadsorbent depends on a number of factors including the higher metal adsorption capacity, specific surface area, user friendly, nature of the material availability and environmental friendliness uses. In this context, comparative adsorption capacities of garden grass and other adsorbents, including activated carbon produced from agricultural wastes (as calculated from the Langmuir isotherm model) for copper are compared in Table 19. It can be seen from these results that the proposed biosorbent from garden grass adsorbs copper from water more than any of the other biosorbents obtained from agricultural wastes and activated carbons. It is also noted that such a biosorbent from garden grass has a higher specific surface area and is arguably more environmentally-friendly due to its non-adjusted pH.

Table 20

The specific surface areas of the garden grass biosorbent

Particle size (μm)	Langmuir Isotherm			Surface area of particle (m^2/g)
	q_m (mg/g)	K (L/mg)	R^2	
150	6.064	0.2036	0.986	90.836
75	8.439	0.3099	0.989	126.404
<75	11.173	0.4587	0.995	167.3620

5 It can therefore be seen that garden grass is a robust reusable and stable biosorbent for metal such as copper and there removal from various materials including water.

Cosmetic product

10

The present invention is not limited to remediating wastewater and the like; it also finds potential application in the field of cosmetics. Many makeups contain undesirable levels of heavy metals that are coated onto a user's skin (in particular, a user's face), absorb into the user's skin – and may subsequently remain in the skin when the makeup is removed either by washing or wiping.

15

The present invention thereby has real potential when incorporated into a cosmetic product, such as a moisturising emollient base, when formulated within a cosmetic “mask” – or even when applied directly to a user's skin. Another possibility is a cosmetic “wipe” comprising one or more of the biosorbents described in relation to the present invention.

20

Furthermore, the constituent/s of the inventive biosorbent – namely, watermelon rind, garden grass and sugarcane bagasse are appealingly “natural” and “organic” to consumers.

25

Although the invention has been described with reference to specific examples it will be appreciated by those skilled in the art that the invention may be embodied in many other forms.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A biosorbent comprising watermelon rind.
- 5 2. A biosorbent comprising sugarcane bagasse.
3. A biosorbent comprising garden grass.
4. A biosorbent comprising at least two components selected from the group
10 consisting of: watermelon rind, sugarcane bagasse and garden grass.
5. A biosorbent according to claim 4, wherein said components are provided in quantities to synergistically enhance biosorptive activity.
- 15 6. A biosorbent according to claim 4 or claim 5, comprising watermelon rind, sugarcane bagasse and garden grass.
7. A biosorbent according to claim 6, wherein said watermelon rind, sugar cane bagasse and garden grass are provided in an approximate 1:1:1 ratio by mass.
20
8. A biosorbent according to any one of the preceding claims, and provided in a dry powdered form.
9. A biosorbent according to any one of the preceding claims, having a pH of about
25 6 to about 7.
10. A biosorbent according to any one of the preceding claims, having a pH of about 6.5 to about 7.
- 30 11. A biosorbent according to any one of the preceding claims, having a pH of about 6.8.
12. A method for removing toxins from a material, said method comprising contacting said material with a biosorbent as defined according to any one of
35 claims 1 to 11.
13. A method according to claim 12, wherein said material is a fluid.

14. A method according to claim 12 or claim 13, wherein said material is an aqueous solution.
- 5 15. A method according to claim 14, wherein said biosorbent is provided in a dosage of between about 0.1 and 5.0 g/L.
16. A method of remediating land, said method comprising contacting said land with a biosorbent as defined according to any one of claims 1 to 11.
- 10 17. A method of adsorbing metal from a material, said method comprising one or more cycles of:
 contacting said material with a biosorbent as defined according to any one of claims 1 to 11; and
15 desorbing said biosorbent.
18. A method according to claim 17, wherein said desorption step comprises contacting said biosorbent with a suitable eluant for a predetermined period.
- 20 19. A method according to claim 17 or claim 18, wherein said method comprises up to 10 cycles.
20. A method according to claim 17 or claim 18, wherein said method comprises up to 30-40 cycles.
- 25 21. A method according to any one of claims 17 to 20, wherein said desorption step (b) comprises contacting said biosorbent with one or more eluants selected from the group consisting of: distilled water, tap water, Milli-Q water, NaOH, HNO₃, HCl, H₂SO₄ and CH₃COOH.
- 30 22. A method according to any one of claims 12 to 21, wherein prior to said contacting step, said biosorbent undergoes a pH pre-treatment to provide said biosorbent at a substantially neutral pH.
- 35 23. A method according to any one of claims 12 to 21, wherein prior to said contacting step, said biosorbent undergoes a pre-treatment comprising contacting said biosorbent with NaOH for a predetermined period.

24. A method according to any one of claims 12 to 23, wherein said biosorbent has a pH of between about 6 and about 7.
- 5 25. A method according to any one of claims 12 to 24, wherein said biosorbent has a pH of about 6.8.
26. A method for adsorbing metals from a material, said method comprising contacting said material with a biosorbent as defined according to any one of
10 claims 1 to 11.
27. A method according to any one of claims 12 to 26, wherein said metals comprise copper, zinc and lead, either alone or in combination.
- 15 28. A method according to any one of claims 12 to 27, wherein said material is water or wastewater.
29. A cosmetic formulation for topical application, said formulation comprising a biosorbent as defined according to any one of claims 1 to 11.
20
30. Use of at least one of watermelon rind, sugar bagasse and garden grass in the preparation of a biosorbent for removing toxins from a material, adsorbing metal from a material and/or remediating wastewater.
- 25 31. A method for the preparation of a biosorbent as defined according to any one of claims 1 to 11, said method comprising the steps of:
obtaining at least one component selected from the group consisting of:
watermelon rind, sugarcane bagasse and garden grass;
drying said at least one component; and
30 grinding said at least one dry component.
32. A method according to claim 31, further comprising a washing step prior to drying said at least one component.
- 35 33. A method according to claim 31 or claim 32, further comprising storing said ground biosorbent in a dry environment such as in a desiccator.

- 31 -

34. A method according to any one of claims 31 to 33, further comprising a pH pre-treatment step, thereby to provide said biosorbent at a substantially neutral pH.
35. A method of removing heavy metals from a user's skin, said method comprising
5 administering to said user an effective amount of a biosorbent as defined according to any one of claims 1 to 11, or of a cosmetic formulation as defined according to claim 29.

FIGURE 1

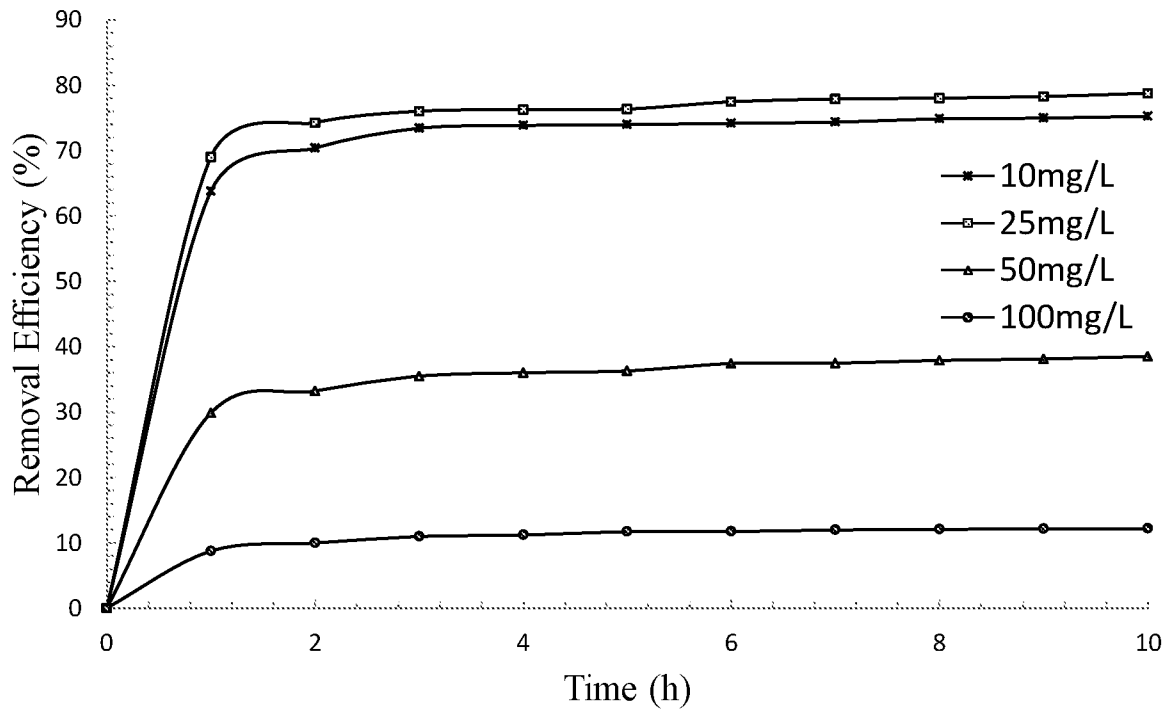


FIGURE 2

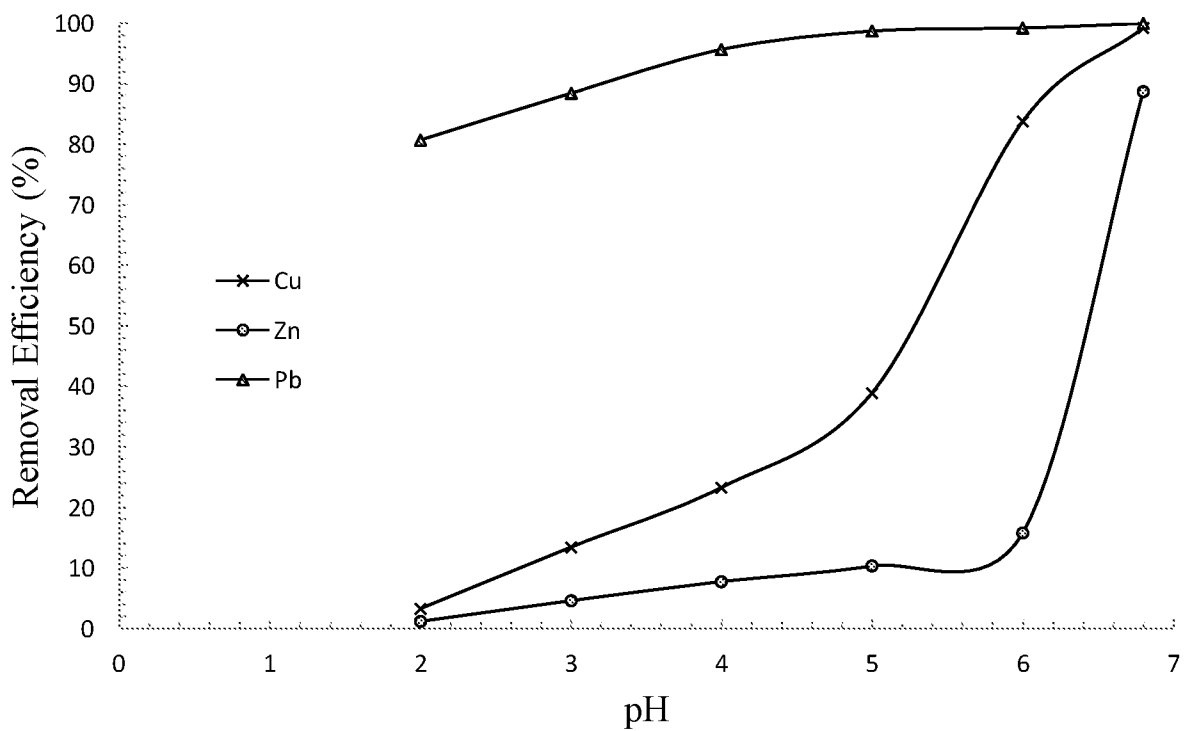


FIGURE 3

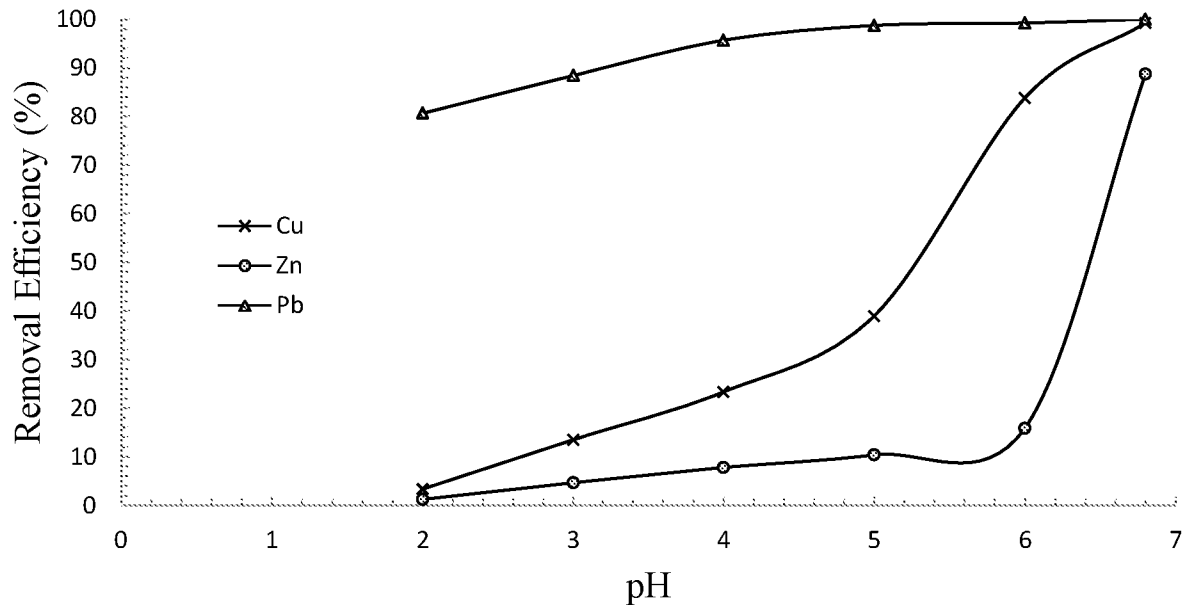


FIGURE 4

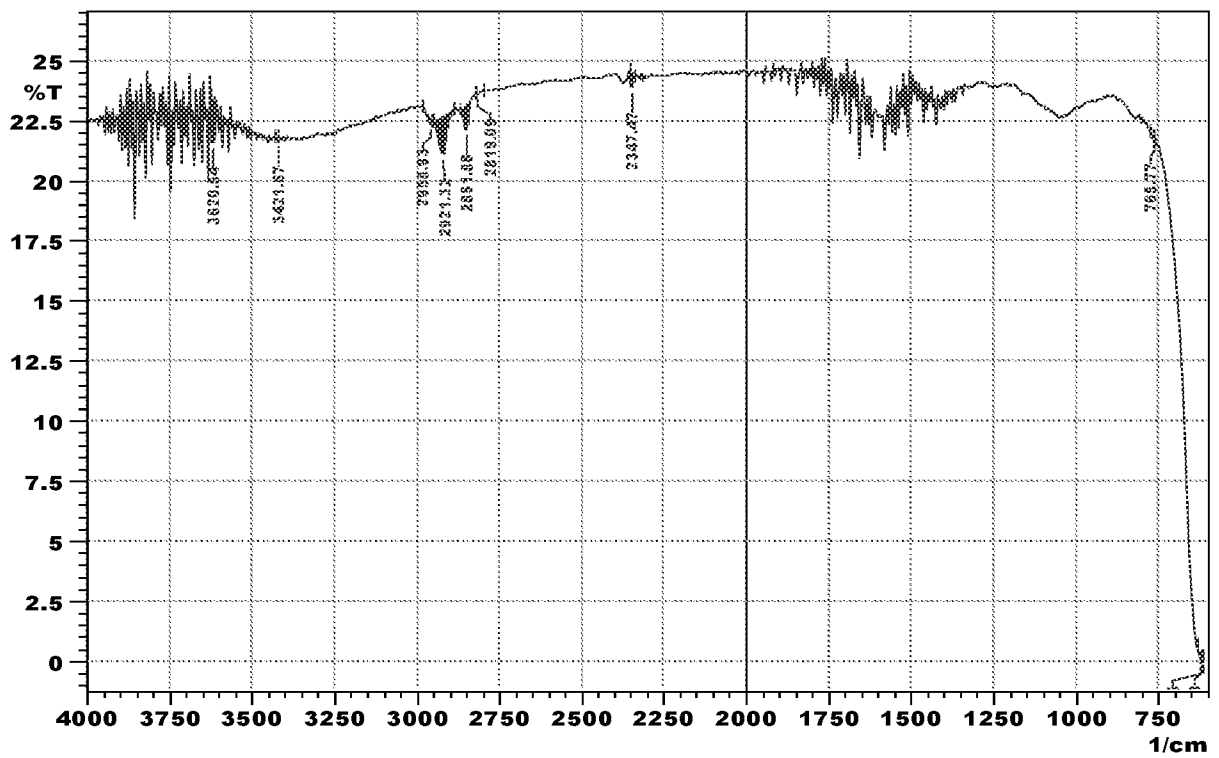


FIGURE 5

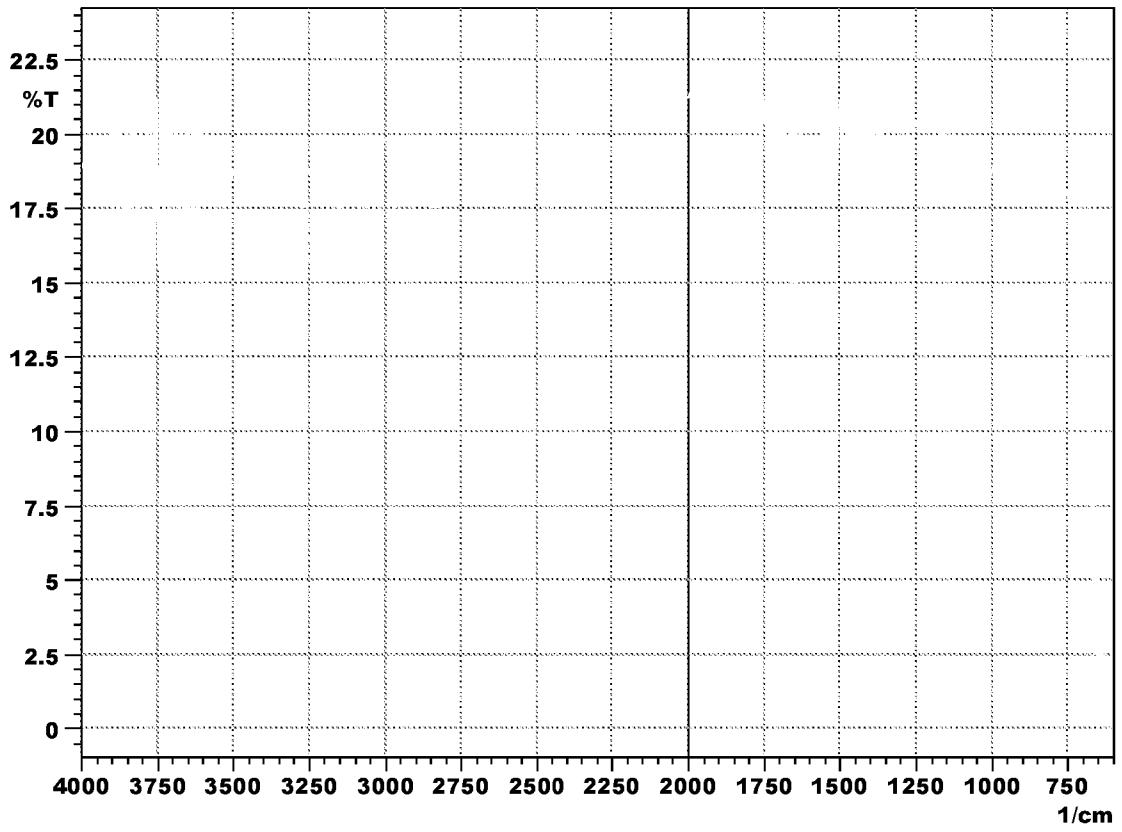


FIGURE 6

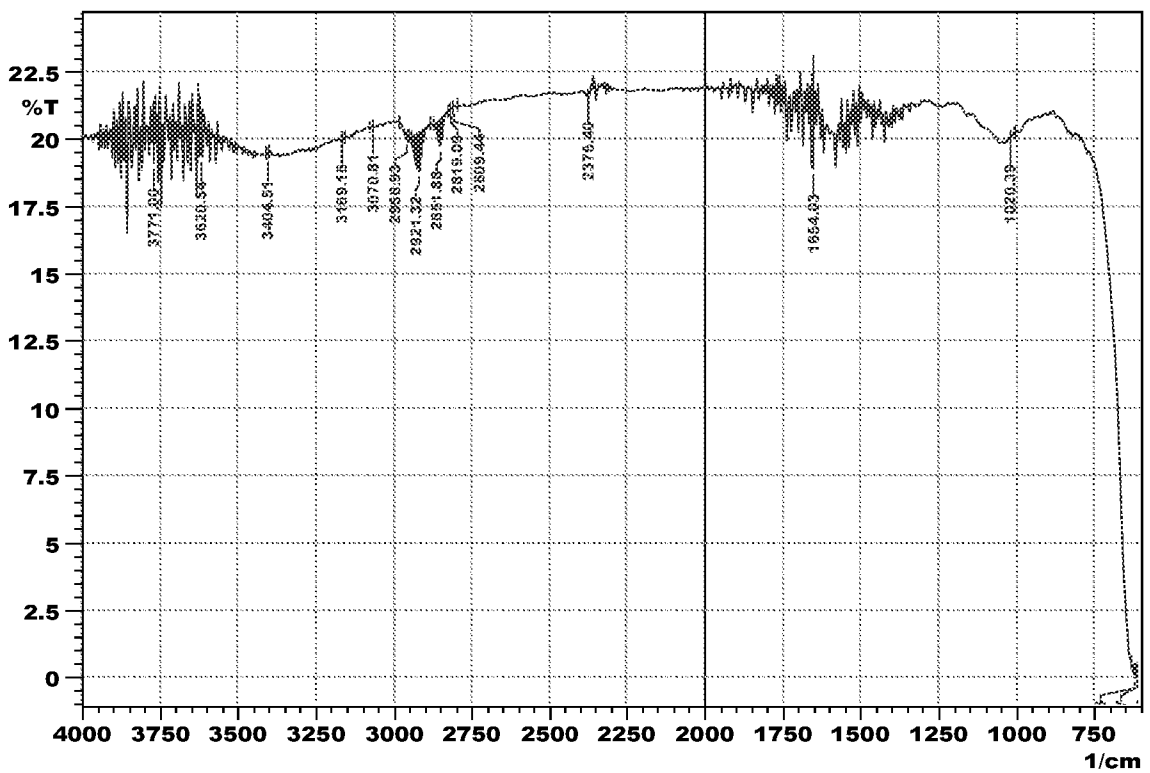


FIGURE 7

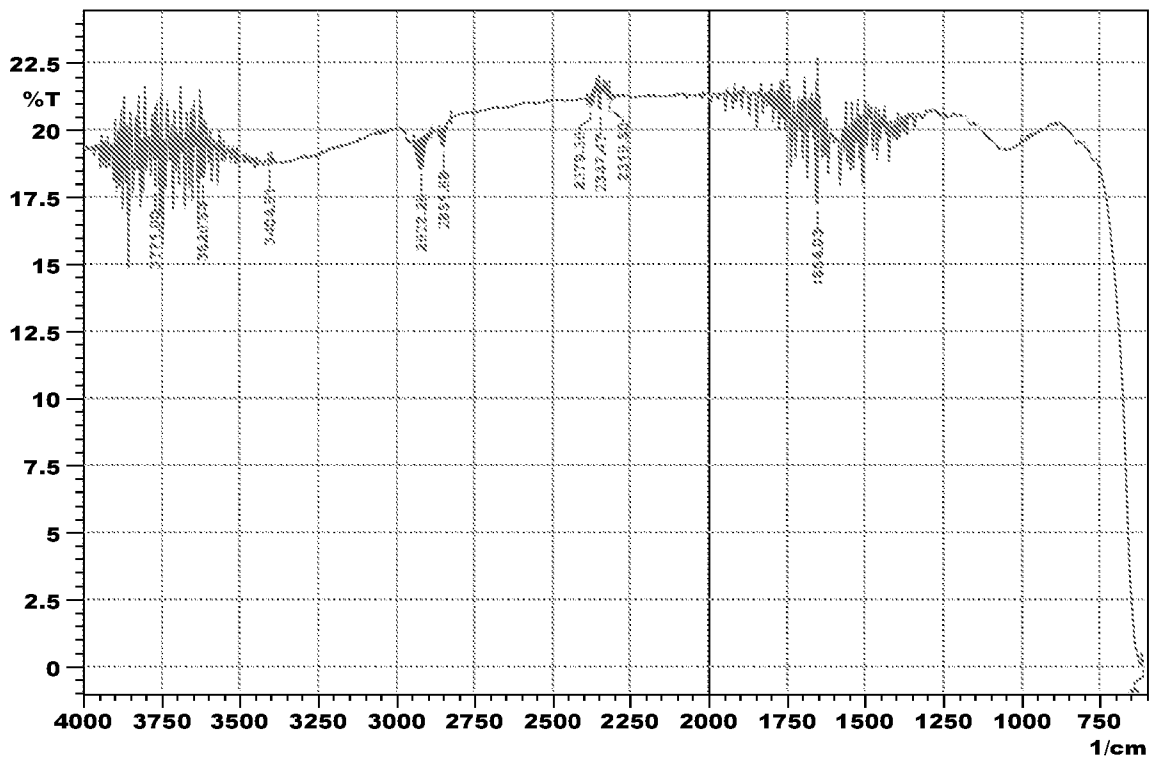


FIGURE 8

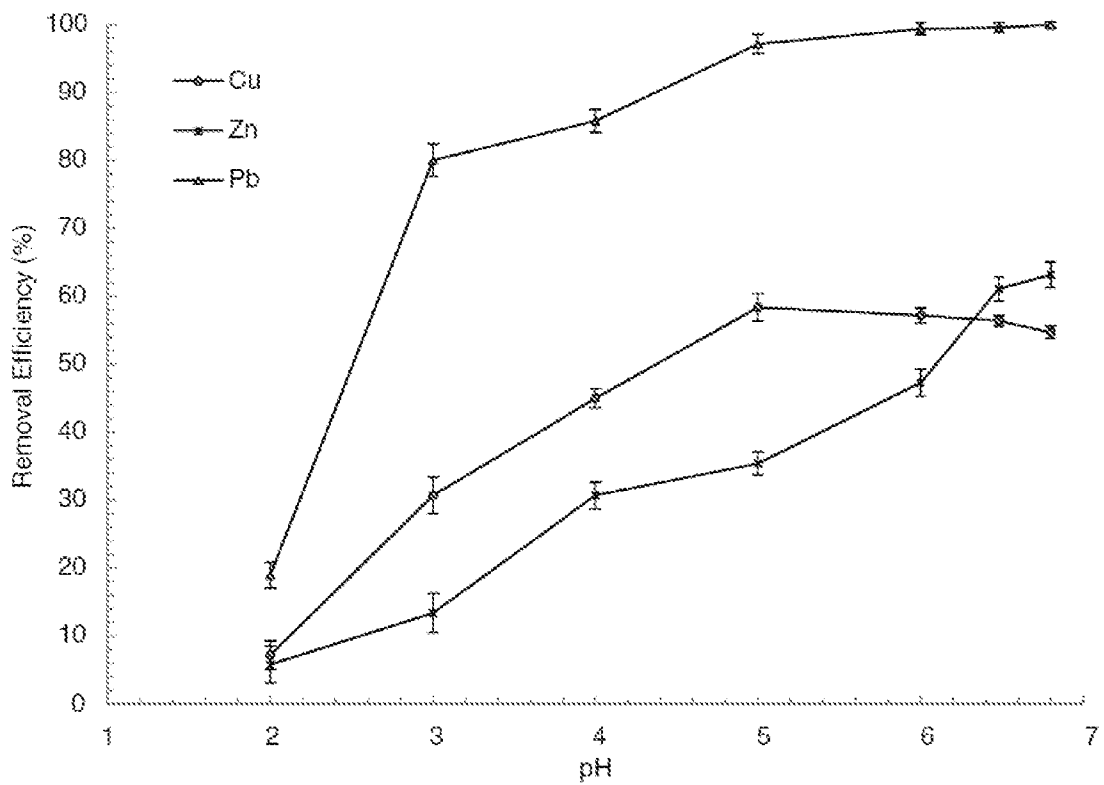


FIGURE 9

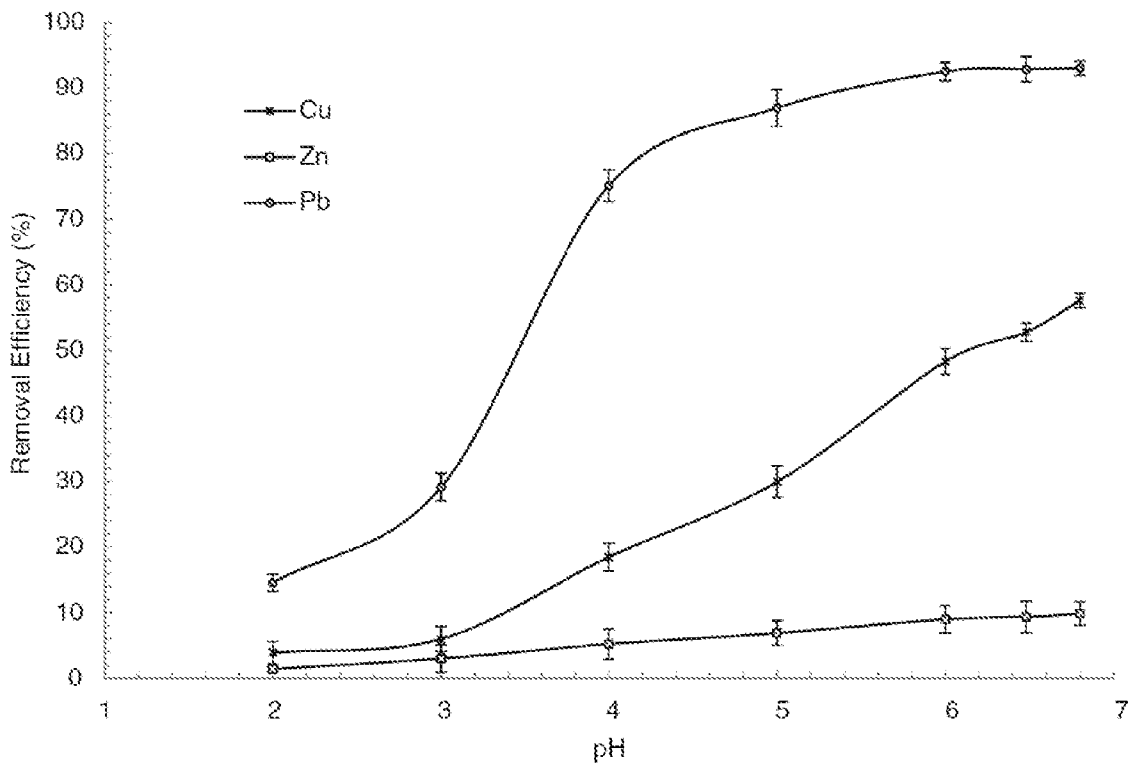


FIGURE 10A

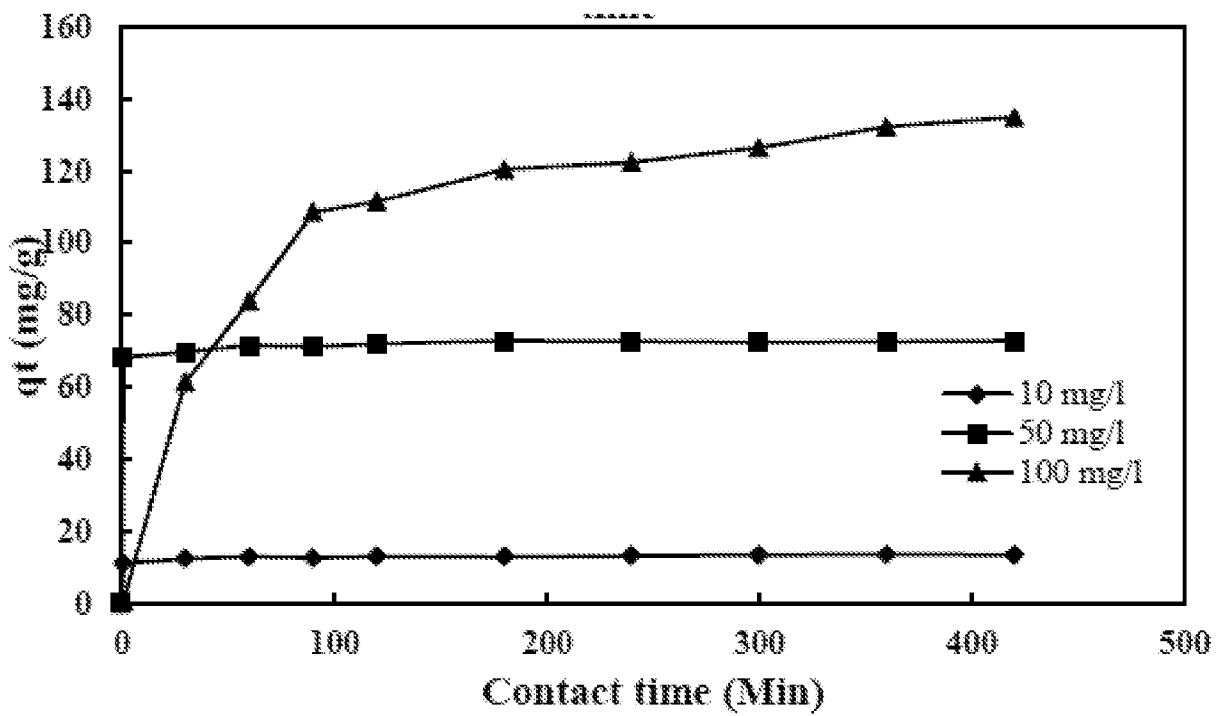


FIGURE 10B

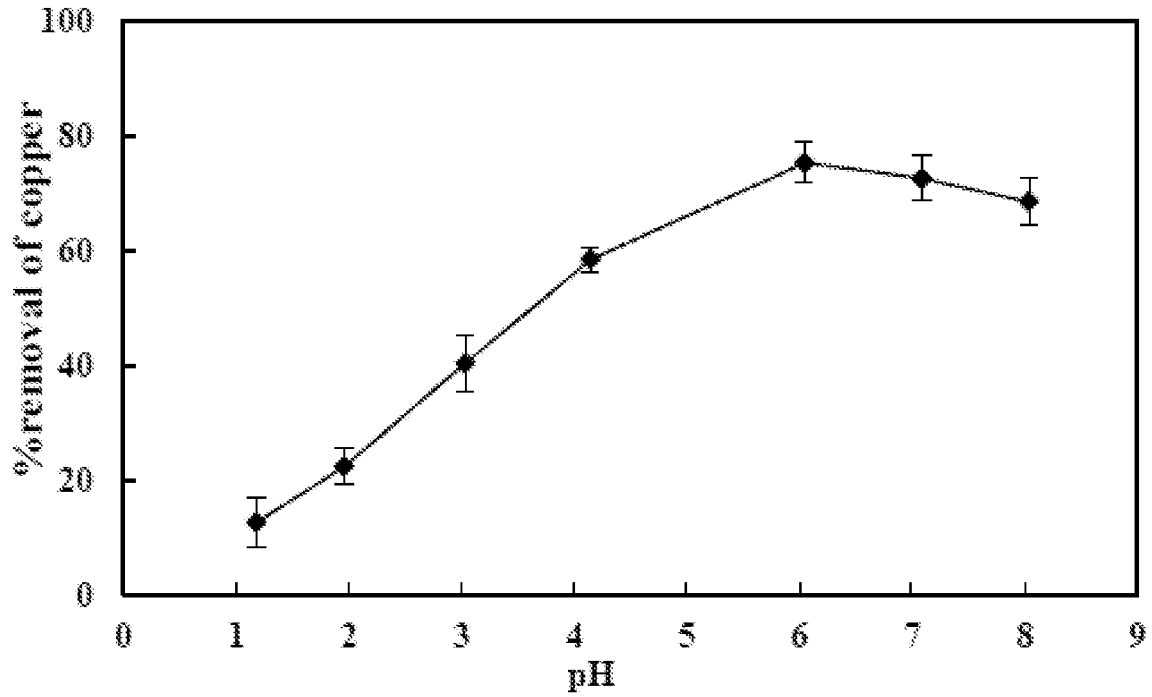


FIGURE 10C

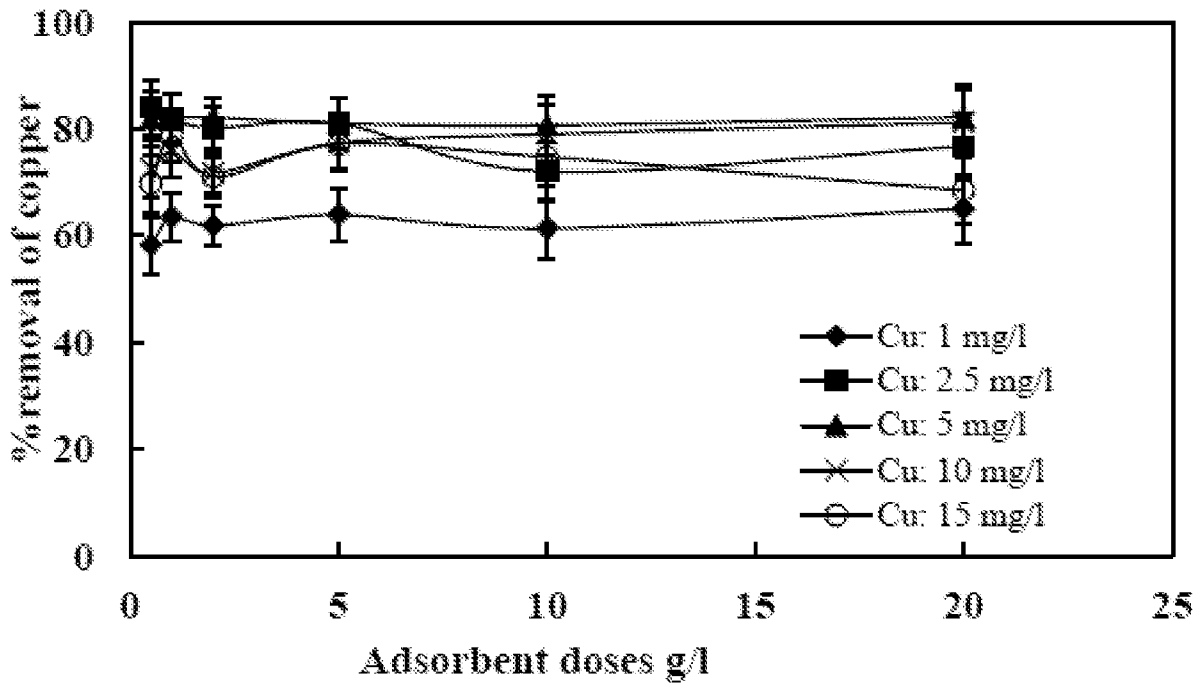


FIGURE 10D

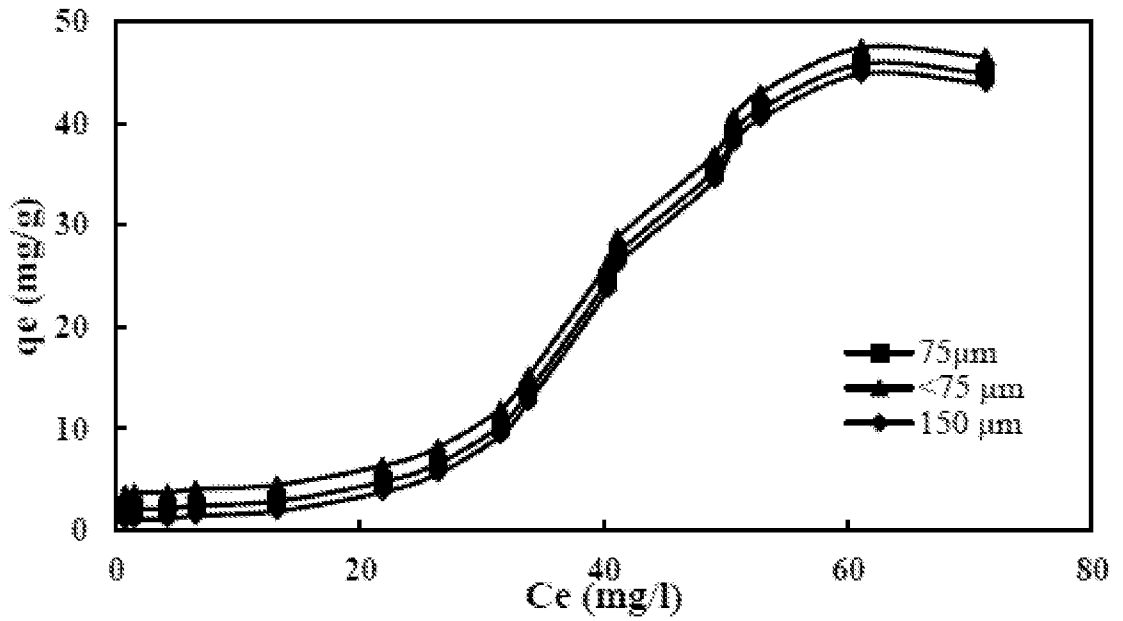


FIGURE 11A

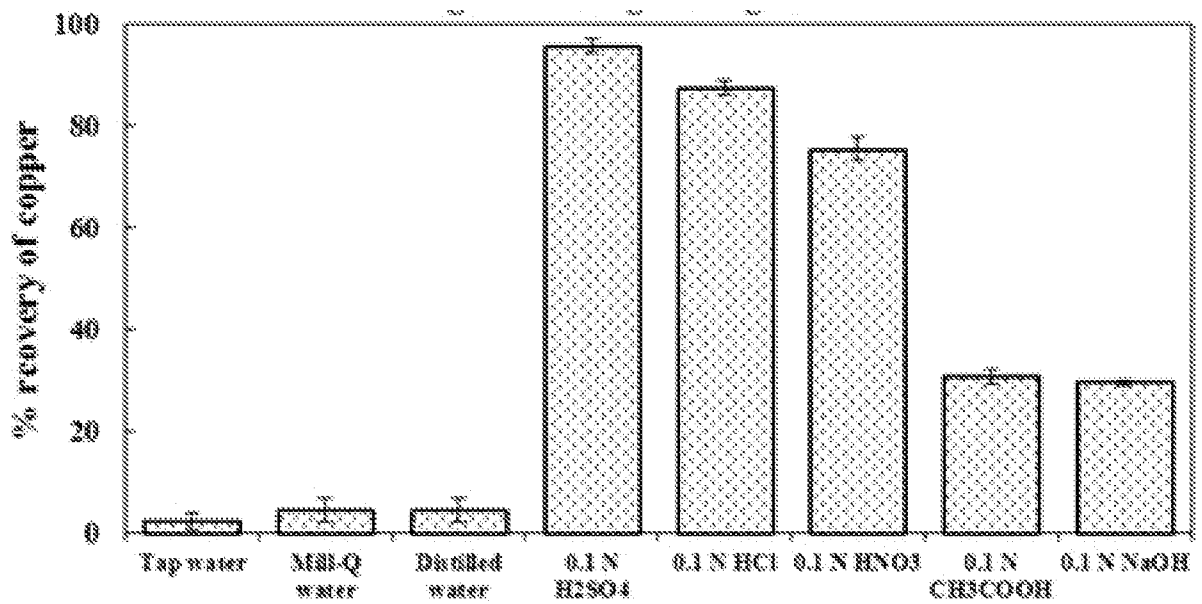
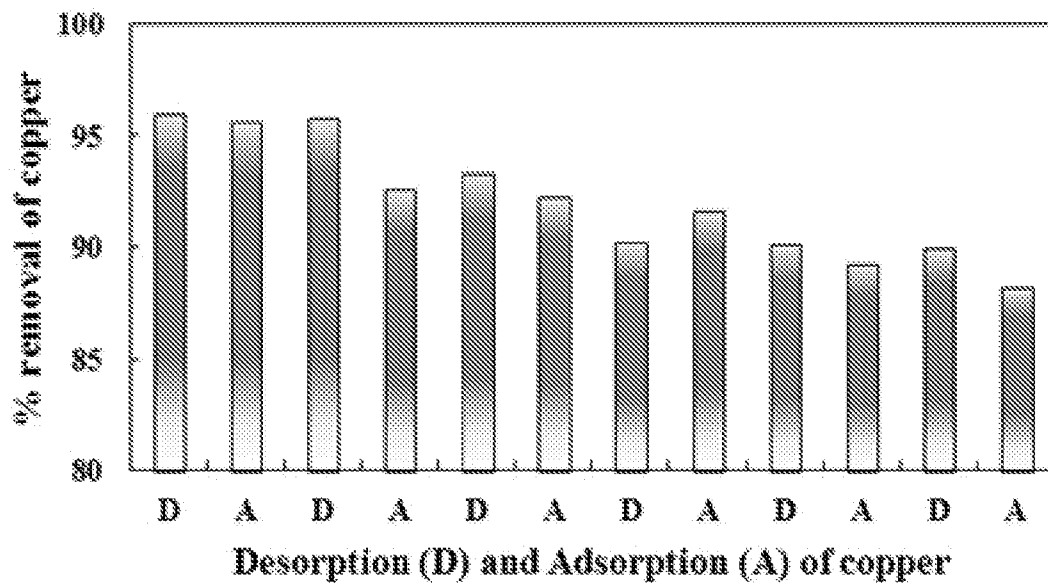


FIGURE 11B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2013/000782

A. CLASSIFICATION OF SUBJECT MATTER

C02F 1/28 (2006.01) C02F 3/32 (2006.01) B09C 1/10 (2006.01) A61Q 19/00 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC: IC/CC (B01J20/22/LOW, B01J20/30/LOW, C02F1/28, C02F1/62, C02F1/286, C02F1/288, C02F2101/20, C02F2303/16, C02F3/32, C22B3/24, B01D15/00, B09C1/10/LOW, A62D3/00, A61Q19, A61K36) AND KEYWORDS (WATERMELON, BAGASSE, GRASS, BIOSORBENT, SUGARCANE, METAL AND SIMILAR WORDS). ESPACENET AND GOOGLE SEARCH: KEYWORDS: WATERMELON, BAGASSE, GRASS, BIOSORBENT, SUGARCANE AND SIMILAR TERMS.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:		
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
6 November 2013Date of mailing of the international search report
08 November 2013

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Telephone No. 0262832535

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2013/000782
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RU 2221639 C1 (AGRIC ACAD MEAT-DAIRY LIVESTOCK BREEDING) 20 January 2004 Abstract (English abstract obtained from WPI database)	1, 4, 6, 8, 12, 13, 14, 17- 23, 26, 27, 31- 34
X	GB 1445754 A (ELEPON KAKOKI CO) 11 August 1976 example 6, table 5, page 1 lines 30 - 47, page 1 line 61, claims 1- 5	2, 8, 12 - 14, 26 - 28 and 30
X	CN 102464376 A (CHANGZHOU YAHUAN ENVIRONMENTAL PROTECTION TECHNOLOGY CO LTD) 23 May 2012 Abstract (English abstract obtained from WPI database); Claims 1 and 4 (claims and description obtained from Google translation)	3, 9 -15, 24 -28 and 30
X	USN 6738815 A (US GOVERNMENT & US SEC OF AGRIC) 24 September 1985 Abstract (English abstract obtained from WPI database).	3
X	JP 2001-276804 A (TAIHEIYO CEMENT CORP) 09 October 2001 Abstract (English abstract obtained from WPI database)	3 and 16
X	CN 101204415 A (TIANJIN ZHONGBAO PHARM CO LTD) 25 June 2008 Abstract (English abstract obtained from WPI database).	3, 29 and 35

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

Supplemental Box**Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1, 4 (in part), 30 (in part) and their dependent claims are directed to a biosorbent comprising watermelon rind. The feature of biosorbent comprising watermelon rind is specific to this group of claims.
- Claims 2, 4 (in part), 30 (in part) and their dependent claims are directed to a biosorbent comprising sugarcane bagasse . The feature of biosorbent comprising sugarcane bagasse is specific to this group of claims.
- Claims 3,4 (in part), 30 (in part) and their dependent claims are directed to a biosorbent comprising garden grass . The feature of biosorbent comprising garden grass is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2013/000782

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
RU 2221639 C1	20 Jan 2004	None	
GB 1445754 A	11 Aug 1976	DE 2357696 A1	06 Jun 1974
		DE 2357696 B2	06 Nov 1975
		FR 2206977 A1	14 Jun 1974
		FR 2206977 B1	01 Oct 1976
		GB 1445754 A	11 Aug 1976
		JP S4973846 A	17 Jul 1974
CN 102464376 A	23 May 2012	None	
USN 6738815 A	24 Sep 1985	None	
JP 2001-276804 A	09 Oct 2001	None	
CN 101204415 A	25 Jun 2008	None	

End of Annex